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Volume 33 *Number 7*

JUL 22 1943

Phytopathology

AN INTERNATIONAL JOURNAL

July 1943

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter

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PARTICLE SIZE OF SULPHUR AND COPPER FUNGICIDES IN RELATION TO APPLE SCAB AND CEDAR-APPLE RUST CONTROL

J. M. Hamilton, D. H. Palmiter, and G. L. Mack[1]

(Accepted for publication October 22, 1942)

Within the past decade, the wettable sulphur fungicides, in both dry and paste forms, have steadily gained favor with fruit growers. They have in large measure supplanted lime-sulphur in commercial spray practice, because of the reduction in spray injury and increased yields of fruit.

Field tests with these proprietary wettable sulphur materials have demonstrated some to be superior to others in their effectiveness in controlling apple scab, *Venturia inaequalis* (Cke.) Wint. (9), and apple rust, *Gymnosporangium juniperi-virginianae* Schw. (10). During the course of these tests, the senior writer became convinced that the outstanding property of these products that could explain their differences in disease control was the size of the sulphur particles.

As early as 1913, Blodgett (2), working with sulphur dusts, pointed out that fine particles adhered to foliage more firmly than did coarser ones. Wilcoxon and McCallan (19), also, demonstrated in laboratory experiments on glass slides that fine-particle sulphur was inherently more toxic to germinating spores than an equal weight of coarse-particle sulphur. But the practical problem of correlating particle size to disease control in the orchard was complicated by the fact that all wettable sulphur fungicides necessarily contain adjuvants that markedly affect their resistance to weathering and often obscure the effects attributable to particle size. Further, the make-up of proprietary spray materials is subject to change from one season to the next.

In order to correlate particle size and disease control, it was necessary to eliminate the interfering effects of (1) varied and secret methods of manufacture, (2) the inclusion of different kinds and amounts of wetting agents, dispersing agents, and other ingredients. In 1937, large samples of a wettable sulphur and an insoluble copper fungicide were obtained whose compositions were known and were alike in all respects, except size of particles (11). The procurement of this material made exact testing possible.

The average particle sizes of these materials and of some of the more common commercial sulphur fungicides were determined and correlated with the results of disease control experiments obtained from their use in laboratory, greenhouse, and orchard.

[1] The writers are indebted to L. O. Weaver for assistance in conducting the greenhouse experiments and in the preparation of the manuscript.

Grateful acknowledgment also is due to R. C. Roark of the Insecticide Laboratory, U. S. Dept. Agr., Bureau of Entomology and Plant Quarantine, to the Stauffer Chemical Company, New York City, and the Micronizer Processing Company, Moorestown, New Jersey, for their cooperation.

Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 531, November 7, 1942.

It is interesting to note that during the course of the studies reported herein, manufacturers have made concerted efforts to improve the effectiveness of commercial, powdered, fungicidal materials by grinding them finer and finer. In 1930, Streeter and Rankin (16) examined 6 representative commercial sulphur materials which varied between 12 and 30 microns for the diameter of the particle of average specific surface,[2] while in 1941, Gooden (6) reported that 42 samples varied between 5 and 25 microns for the same diameter. Values reported in this communication extend the lower limit of average particle size to 2 microns in commercial dry wettable sulphur fungicides. Thus, in 12 years, the lower limit of average particle size has been reduced sixfold, while the upper limit has remained practically stationary.

METHODS OF MEASURING PARTICLE SIZE

The fineness of particles usually has been expressed as a function of the diameter, although several authors have pointed out the inadequacy of that expression when the individual particles are not all the same size and shape. Green (8) has shown that there can be no generalized expression for average particle size, and has discussed 6 different diameters that are equal only when every particle has the same size and shape. In a non-uniform powder, they vary as follows:[3]

$$d_4 > d_3 > d_2 \begin{matrix} < \\ = \\ > \end{matrix} D > \triangle > d_1$$

With a polydisperse material such as ground sulphur, a 4- or 5-fold variation between the smallest and largest of these diameters is not uncommon. It was, therefore, extremely important to choose that particular diameter best suited for denoting the particle size of fungicides.

The property of a powdered fungicide apparently most closely related to the degree of disease control obtainable was the specific surface area. Accordingly, the surface average diameter, d_3, was selected for designating the relative degree of fineness of fungicidal materials. The surface average diameter can be expressed mathematically as

$$d_3 = \frac{\sum nd^3}{\sum nd^2}$$

where n is the total number of particles in the sample and d is any diameter of the individual particle. More explicitly, the surface average diameter may be defined as the diameter of a sphere whose specific surface is the same as that of the sample. This idealized sphere need not exist in fact; *i.e.*, a sphere may be used to represent the average diameter of particles, none of which are spherical in shape. It will be seen that in any measurement of particle size, some assumption as to the general shape of the particles must be made.

[2] The writers have calculated these figures from the original data of Streeter and Rankin. The average specific surface is defined as the specific surface of the sample as a whole and not the arithmetic average of the specific surfaces of the individual particles.

[3] The symbols employed throughout this discussion are the same as those adopted by Green (8).

Two other of the diameters given by Green (8) have been used by previous workers to express the particle size of fungicidal materials. These are the *arithmetic mean diameter*, d_1, and the *diameter of the particle of mean weight*, D. They are calculated by means of the following equations:

$$d_1 = \frac{\Sigma nd}{\Sigma n}$$

$$D = \frac{\sqrt[3]{\Sigma nd^3}}{\Sigma n}$$

While it was not considered that these latter two diameters had the fundamental significance of the surface average diameter, they were included in a part of the particle-size measurements for the sake of comparing the different methods. No attempt was made to convert these different diameters to a common basis because of the uncertain value of the shape factor.

Four completely independent methods were employed for the determination of particle size. These were the sedimentation method (1), the air-permeation method (7), the direct microscopic method, and the microscopic-projection method (8, 13). The agreement between the results is remarkably good when it is considered that the properties which were actually measured were in order (1) a settling velocity, or rate of fall of particles through a liquid medium, (2) a permeability, or resistance to flow of air through a packed bed of particles, (3) the diameters of a number of particles, and (4) the number of particles of known weight that occupy a given area (Table 1). Lack of absolute agreement can be ascribed principally to differences inherent in the measurements and calculations. The possibility of changes in the samples themselves cannot be overlooked, since these experiments extended over a period of several years.

In general, it was considered that the *sedimentation method* using the Andreasen pipette gave the most reliable results (1). By this method, the distribution of particle sizes is obtained directly, so that the results may be subjected to critical analysis and checked by microscopic observation. In a few cases, agglomeration produced particles of irregular shape. These were assumed to settle as cubes according to the procedure of Andreasen, but otherwise, all particles were assumed to settle as spheres. This method has a most important advantage over other sedimentation methods in that any inert material, which also settles out, is not determined. But the presence of suspending agents, such as bentonite, can cause a lower reading by interfering with the normal sedimentation of the sulphur particles. Thus, the average particle size of Kolospray is probably somewhat larger than the apparent value of 7.7 microns (Table 2).

A second useful method was found to be the air permeation procedure as modified by Gooden and Smith (7). While the apparatus is somewhat complicated, the method is very rapid and well suited to routine work. The agreement between results obtained upon the same materials in two different laboratories is good (Table 1), and a further comparison of the sedimenta-

tion and air permeation methods shows remarkably concordant results (Table 2).

The air-permeation method can give low readings with materials which contain an insufficient number of very fine particles to completely fill the voids between the larger particles. The reason that the air permeation method is somewhat more accurate than the original liquid permeation method of Carman (3) is that the liquid acts as a lubricant, allowing the finer material to pack tightly into the voids between the larger particles. McMillen (15) has shown that a membrane consisting of a polydisperse powder has a smaller average pore diameter, when packed wet than when packed dry. Segregation of the finer particles is thus considerably reduced

TABLE 1.—*Comparison of results obtained from various methods of measuring particle size*

Material	Year	Surface average diameter, d_3			Number average diameter, d_1	Weight average diameter, D
		Sedimentation	Air permeation		Direct microscopic[a]	Microscopic projection
			A	B[b]		
Special sulphur (fine)	1937	.....	3.5	4	2	7
Micronized	1940	3.6	3.7	5	3	.....
Special sulphur (medium)	1937	.....	6.6	7	6	10
Special sulphur	1937	.....	10.3	12	18	13
Mike	1940	5.3	5.5	6	5	.....
Magnetic 70 (dry)	1940	5.2	5.8	7	5	.....
Magnetic-Spray	1940	8.3	9.7	.....	8	.....
Sulcoloid	1937	.....	.....	10	11	9
Z–O[c] (fine)	1938	1.6	1.4	1	6	1
Z–O (medium)	1938	10.0	8.2	6	10	7
Z–O (coarse)	1938	13.3	10.0	8	20	8

[a] The results listed in this column were obtained in the control laboratory of the Micronizer Processing Co., Moorestown, N. J.

[b] The results listed in this column were obtained in the insecticide laboratory of the U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, under the direction of R. C. Roark.

[c] Z–O is a proprietary copper ammonium zeolite.

but not entirely eliminated by packing the powder dry. Uniform results are obtained by using the powder compactor designed by Gooden (5). This packing effect probably accounts for the low results obtained by the air permeation method for Microsulphur and Sulcoloid (Table 2), and for the medium and coarse samples of copper zeolite (Table 1).

Conversely, the air-permeation readings are too high for Magnetic-Spray and Kolospray (Tables 1 and 2). This is no criticism of the method, since these values are correct under the conditions of the measurements; but these materials contain aggregates, formed in the process of manufacture, which function as single particles in air and are dispersed into many particles in water. The fused bentonite sulphur material known as Kolofog is an ex-

treme example of this effect, since the average particle diameter in air is over 100 microns, while, in water, it is less than 1 micron. The air permeation method is, therefore, better suited to the determination of the particle size of dusts than of spray materials.

The microscopic measurement of particle size is considered by many to yield the most reliable results because it is visual and direct, and conforms more closely to the usual method of determining the size of larger objects. But, for irregular objects, the results are always too large, since the particles will tend to settle so that the two horizontal dimensions measured will be larger than the vertical dimension, which is not. This effect is apparent

TABLE 2.—*The particle size of some commercial wettable sulphur fungicides as determined by sedimentation and air-permeation methods*

Brand name[a]	Surface average diameter		Per cent by weight of particles less than 4 μ in diameter	Sulphur content	Manufacturer
	Sedimentation	Air permeation			
	Microns	*Microns*		*Per cent*	
Koppers Dry Wettable	2.0	2.2	89.6	91.0	Koppers Products Company
Micronized Sulphur	3.6	3.7	46.5	93.4	Micronizer Processing Co.
Mike Sulphur	5.3	5.5	21.2	92.5	Dow Chemical Company
Sulforon	6.6	6.6	11.4	98.0	DuPont de Nemours & Co.
Micro-Spray	6.7	5.9	9.7	92.5	General Chemical Company
Kolospray	7.7	11.2	8.2	83.5	Niagara Sprayer & Chem. Co.
Magnetic-Spray	8.3	9.7	7.4	98.5	Stauffer Chemical Company
Sulfix	8.8	8.8	7.0	97.3	Sherwin-Williams Company
Microsulphur	10.3	6.3	4.3	82.0	Chipman Chemical Company
Dritomic	12.2	13.2	0.8	82.0	General Chemical Company
Sulcoloid (1936)	14.2	12.6	1.2	93.0	Ansbacher Chemical Company

[a] 1940 samples unless otherwise stated.

with the coarsest special sulphur and all of the copper samples (Table 1). Even if these values be multiplied by the usual shape-correction factor of $\frac{2}{3}$, agreement with the results of other methods is not good, since d_1 should be much less than d_3 with the finest copper zeolite. The very high figure obtained for d_1 may have been due to the fact that many of the finer translucent crystals of the zeolite were passed over in the counting. The photomicrograph of this material indicates that this might easily have been the case (Fig. 1).

To reduce the enormous amount of labor required to measure the particles in the finer materials, a method of treating the distribution count has been devised, so that only a relatively small number of particles need be counted (13, 17). By assuming that the Poisson size distribution occurs, and that the particles are spherical, a relatively simple calculation yields the

diameter of the particle of average weight (last column, Table 1). The results are in good agreement with those obtained.

Photomicrographs of spray residues of the different materials, when deposited as fine, evenly spaced, particulate drops upon glass slides, with a vertical sprayer, confirm the results of the particle size measurements. (*Cf.*

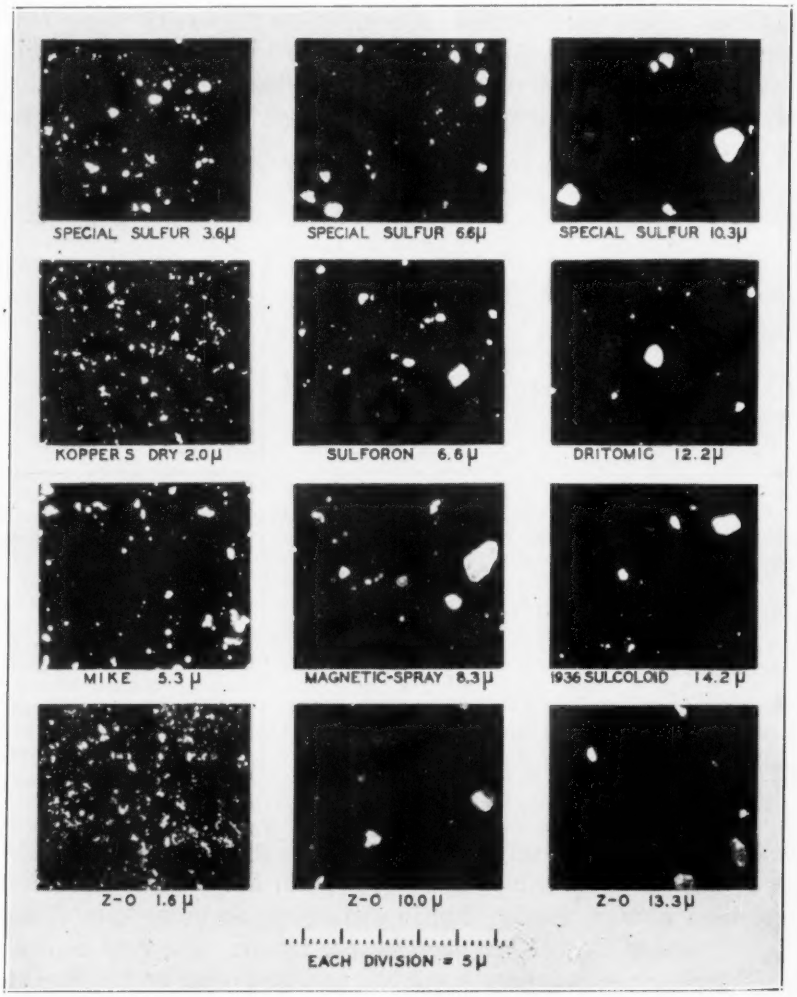

FIG. 1. Representative microscopic fields of sulphur and copper ammonium zeolite particles deposited upon glass slides in finely atomized even spaced particulate drops. The weight of all the particles in each area is the same. Duplicate slides were found by chemical analysis to contain 7.0 ± 0.5 micrograms of sulphur or copper ammonium zeolite per sq. cm.

Tables 1 and 2 with Fig. 1.) Since the fields of view are small, great care was taken to select a field truly representative of the material as a whole. A consideration of these photographs suggests that the larger average particle sizes result not so much from the presence of very large particles

as from the relatively small number of particles less than 4 or 5 microns in diameter.

While the differences between certain values have been discussed at length, it should be emphasized that the agreement between determinations in different laboratories on the materials of different ages and conditions of sampling still is remarkably close. From a practical point of view, a variation of 10 to 20 per cent in the average particle diameter of a wettable sulphur would scarcely be noticed in field experiments. Furthermore, the true particle size in water may not be the same as the effective particle size in an actual spray mixture. It is known that lime, lead arsenate, and many oil stickers flocculate sulphur particles into large agglomerates.

STUDIES OF DISEASE CONTROL

Orchard Experiments with Apple Scab

Methods. The experiments were conducted in mature, vigorous orchards in which the primary inoculum of apple scab was considered moderate to heavy. Single-tree plots were replicated from 2 to 6 times. A modern power sprayer was used, operated at 400 lb. pressure, with a single gun. The spray materials were washed, with the gun, through a screen into the tank. The same brand of arsenate of lead, which contained no wetting agent, was used in all the tests. At harvest, the scab-infected fruit was divided into 3 classes; slight, medium, and bad. Slight scab included those fruits that had scab spots affecting a total area up to $\frac{1}{4}$ inch in aggregate diameter, medium scab up to $\frac{3}{4}$ inch, and bad scab more than $\frac{3}{4}$ inch.

Seasonal and Orchard Conditions. Rainfall in the Hudson Valley varies considerably, not only from year to year but also from one locality to another in the same year. The amount of primary inoculum carried over in the old leaves also varies from orchard to orchard, depending on the previous disease history of the individual orchard. Therefore, it seems advisable to give a brief summary of the rainfall and inoculum facts concerned in these experiments (Table 3).

Series 1. Heavy primary inoculum and numerous prolonged rains made conditions ideal for a fungicide test. Some leaf infections occurred in all plots during bloom, and these became a source of secondary inoculum from which the trees had to be protected during the rest of the season. Fruits on nonsprayed Cortland trees became so heavily infected early in the season that they all dropped during June and July. Since all materials were applied at low concentrations, to accentuate any differences in the control of apple scab, it was necessary to apply 10 sprays instead of the 7 usually recommended.

Series 2. Primary inoculum and rainfall were considered average. Lack of protection during the abnormally long bloom period provided a source of secondary inoculum and a severe test for the fungicides during the rest of the season. Seven sprays were applied in this orchard.

Series 3 and 4. The orchard had only a moderate primary inoculum. The season was early and the trees passed through the first 4 spray applica-

tions with little chance for infection. Heavy rains occurred during the remainder of the season, and only the retentive materials were able to withstand this drastic washing.

Series 5. The orchard had a heavy carry-over of scab. Dry weather early in the season prevented development of scab on sprayed trees until July, but two heavy rains in August gave the fungus a chance to cause late fruit infection.

Treatments and Results with Sulphur Materials. The field experiments were designed primarily to determine the fungicidal values of the specially prepared sulphurs in which particle size was the only variable factor (Table 4, Ser. 1, 3, 5). Included, however, were commercial brands varying in particle size, but in which the effects are complicated by other factors such as shape of sulphur particles, differences in age of material, presence of adjuvants, and methods of adding wetting agents (Table 4, Ser. 2 and 4).

TABLE 3.—*Record of environmental factors that influenced apple scab development in experimental orchards of the Hudson Valley, 1937–1939*

Year and series[a]	Location	Leaves with perithecia[b]	Rains	Seasonal rainfall[c]	Infection periods[d]
		Per cent	*No.*	*Inches*	*No.*
1937 Ser. 1	Fishkill	71	43	29	11
1937 Ser. 2	Clermont	24	16	14	9
1938 Ser. 3 and 4	Rhinebeck	38	35	24	9
1939 Ser. 5	Modena	80	19	4	5

[a] Series numbers identical to those in table 4.
[b] Percentage of overwintered leaves bearing perithecia. In each series, ascospores were mature by the delayed dormant stage of tree development.
[c] Rainfall, green-tip to harvest.
[d] Rain periods of sufficient duration for scab infection.

The data show, in every experiment, that the relative value of the wettable sulphurs in scab control was in inverse proportion to the average diameter of the sulphur particles. This all-important fact is true, not only for the special sulphurs but also for the commercial brands. Factors that may accentuate or obscure the differences in the degree of disease control obtainable with different particle-size sulphurs are seasonal weather conditions, inoculum potential, timing of sprays, varietal susceptibility, and concentration of toxicant.

In 1937, a wet year, commercial control of scab was obtained on McIntosh apples sprayed with 3.7 micron sulphur, while there was over 50 per cent scabby fruits in plots sprayed with 6.6 and 10.3 μ sulphur (Ser. 1). In 1939, a dry year, only the plot sprayed with the 10.3 μ sulphur failed to give commercial control (Ser. 5). The effect of particle size evidently is accentuated under conditions favorable for scab development.

Proper timing of spray applications tends to minimize the differences in scab control obtained with different particle-size sulphurs (Ser. 4). But in those orchards where scab had become established in the trees early in

the season, large differences in scab control were obtained with different particle-size sulphurs. (*Cf.* Ser. 1 and 2 with Ser. 3 and 4.)

Lowering the concentration of the sulphurs below the point where commercial control could be expected with the better materials further empha-

TABLE 4.—*The size of sulphur particles in relation to control of apple scab*

Series, year, variety, and treatment	Average particle size of sulphur[a]	Total fruit	Percentage of scabbed fruits			
			Slight	Medium	Bad	Total
Series 1[b]—1937						
Delicious						
Special sulphur 4–100	3.7	1277	3.8	3.7	2.8	10.3
	6.6	486	8.2	7.8	4.3	20.3
	10.3	1461	18.0	17.7	14.6	50.3
Cortland						
Special sulphur 4–100	3.7	3510	10.3	5.1	3.9	19.3
	6.6	3453	12.5	11.6	6.4	30.5
	10.3	2014	14.4	20.1	29.8	64.3
McIntosh						
Special sulphur 4–100	3.7	523	6.1	1.9	2.3	10.3
	6.6	268	18.2	9.3	26.5	54.1
	10.3	365	29.6	16.8	19.5	65.9
Series 2—1937						
McIntosh						
Nontreated	.....	7798	1.6	2.9	95.0	99.5
Dow Mike 2–100	5.3	3427	15.4	10.3	3.2	28.9
Magnetic-Spray 2–100	8.3	2954	19.3	21.3	12.0	52.6
Dow Mike 4–100	5.3	4479	9.7	7.3	3.5	20.5
Magnetic-Spray 4–100	8.3	3341	14.4	8.8	3.8	27.5
Sulcoloid 4–100	14.2	3737	22.9	16.8	7.8	47.5
Series 3—1938						
McIntosh						
Nontreated	.....	3031	5.7	10.5	83.6	99.8
Special sulphur 3–100	3.7	2609	4.3	3.5	3.6	11.4
	6.6	4318	5.4	9.6	9.2	24.2
Special sulphur 5–100	3.7	6818	1.7	1.0	1.2	3.9
	6.6	4222	2.2	2.2	1.3	5.7
	10.3	5786	5.6	4.4	2.8	12.8
Series 4—1938						
McIntosh						
Nontreated	.....	3031	5.7	10.5	83.6	99.8
Dow Mike 5–100	5.3	3941	1.4	1.4	1.6	4.4
Magnetic-Spray 6–100	8.3	4413	2.7	4.9	1.8	9.4
Sulcoloid 6–100	14.2	4665	3.3	6.1	3.0	12.4
Series 5—1939						
McIntosh						
Nontreated	.....	7045	12.3	13.7	66.0	92.0
Special sulphur 4–100	3.7	2449	0.4	0.1	0.0	0.5
	6.6	9851	2.3	1.0	0.7	4.0
	10.3	8355	15.0	13.6	6.8	35.4
Special sulphur 8–100	10.3	5415	11.8	6.5	3.7	22.0

[a] Surface average diameter.

[b] Nontreated trees in series 1 were heavily scabbed in June, and all fruits dropped at that time.

sized the greater effectiveness of the materials of smaller particle size (Ser. 2 and 3). Doubling the concentration of the 10.3 micron sulphur did not compensate for the greater toxicity of the 3.7 and 6.6 micron materials (Ser. 5).

Greenhouse Experiments with Cedar-Apple Rust

Methods and Conditions. Potted Medina and Wealthy apple trees of comparable shoot development were selected for each experiment.

The trees were placed on a turntable and given a regulated application of a fungicide spray. These sprayed trees were then allowed to dry over night, and subsequently subjected to periods of artificial "rain" wash on the turntable (12).

For inoculation, the trees were atomized with standardized water suspensions of sporidia of *Gymnosporangium juniperi-virginianae*. Next, they were placed in a moist chamber for 16 hours at 16° C. and then returned to the greenhouse for the incubation period of at least 2 weeks.

Counts were made of the number of lesions on the 3 most heavily infected leaves per shoot, as the index to the effectiveness of the various fungicidal materials.

Treatments and Results with Sulphur and Copper Materials

Summarization of greenhouse tests shows particle size to be the predominant factor in the fungicidal value of both the sulphur and the copper materials (Tables 5, 6, and 7).

The specially prepared sulphur, with different average particle size as the only variable factor, gave control of cedar-apple rust in inverse proportion to the size of the toxic particles (Table 5). A mixture of equal weights of the 3.7 and 10.3 μ sulphur was not far different in effectiveness from the 6.6 μ sulphur (Table 5, Ser. 2).

Ground copper zeolite, with average particle sizes of 1.6, 10, and 13.3 μ, exhibited fungicidal value in proportion to their relative degrees of fineness (Table 7).

Most of the commercial wettable sulphurs, also, adhered to this relationship between particle size and disease control (Table 6). Koppers Dry Wettable (2.0 μ), Micronized (3.6 μ), Sulforon (6.6 μ), Micro-Spray (6.7 μ), Kolospray (7.7 μ), Sulfix (8.8 μ), Microsulphur (10.3 μ), Apple Dritomic (12.2 μ), and Sulcoloid (14.2 μ) were effective in cedar-apple rust control about in that order. However, with Mike (5.3 μ), Magnetic-Spray (8.3 μ), and Kolofog (?), other factors in their composition seemed to obscure the particle size-disease control ratio. The shape of the particles in Mike sulphur and the thoroughness with which these are wet, due to the process of manufacture, may be factors influencing its tenacity and, consequently, its control. Unpublished data show that tenacity of wettable sulphurs tends to increase with age. In the case of Magnetic-Spray, electrical charges and a chemical used in the manufacturing process are claimed to be factors in its effectiveness, but these factors do not account entirely for the irregularities in disease control. Kolofog, a fused bentonite-sulphur containing sulphur particles of colloidal dimensions when dispersed in water, gave excellent control in this type of test (Ser. 5). However, under field conditions, Kolofog has not given consistent disease control presumably because the material

is either too rententive or has too low a sulphur content to be sufficiently redistributed.

THE RELATIONSHIP BETWEEN PARTICLE SIZE, RETENTION, AND INHERENT TOXICITY OF SULPHUR AND COPPER MATERIALS

It has been shown that better control of apple scab and cedar-apple rust usually is obtained with the fungicides of smaller particle sizes. The question arises as to whether the increased control is due to a greater adherence of small particles to the leaves and fruit, to more thorough distribution, or to greater toxicity when proportionately larger surface areas of the toxic

TABLE 5.—*Cedar-apple rust control on potted Medina apple trees sprayed with sulphur of different average particle size*

Series and treatment	Particle size[a]	Av. No. lesions on 3 heaviest infected leaves per shoot	
		2-in. wash[b]	3-in. wash
Series 1			
Special sulphur 5–100	3.5	1	1
	3.7	1	6
	6.6	8	5
	10.3	100 ±	100 ±
Unsprayed	.....	100 ±	.....
Series 2			
Special sulphur 5–100	3.7	1	8
	6.6	30	37
Special sulphur 2½–100)	3.7)	30	60
" " 2½–100)	10.3)		
Unsprayed	.....	80	.....
Series 3			
Special sulphur 5–100 + S. E. C. Oil,[c] 1 pt.	3.7	12	22
	6.6	20	23
	10.3	90	.....
Unsprayed	.....	300 ±	.....

[a] Surface average diameter.
[b] Inches of artificial rain on turntable.
[c] Self-emulsifiable cottonseed oil.

material are exposed. The relative adhesiveness was determined by analyses of the sulphur residues left on sprayed leaves. The effect of particle distribution and inherent toxicity was studied *in vitro* using the inhibition of germination of *Sclerotinia fructicola* spores as an indicator.

Sulphur Residue Analyses

Methods.—The sulphur residue analyses were made from leaf samples taken from sprayed orchard trees after various amounts of rainfall. The leaf samples were taken at random from the lower portions of three or more trees that had been sprayed as evenly as possible after terminal growth had stopped. As far as possible, leaves were selected from the same positions on the terminals. All samples for each series were collected by the same

person, to reduce sampling error to a minimum. Each sample lot consisted of 200 to 300 leaves. A 1-inch disc was cut from each leaf with a hollow steel punch. The discs were oven-dried, ground, and stored dry in fruit

TABLE 6.—*Cedar-apple rust control on potted Medina apple trees sprayed with commercial sulphur fungicides of different average particle size*

Series and treatment	Particle size[a]	Av. No. lesions on 3 most heavily infected leaves per shoot
		0.5 in. wash[b]
Series 1		
Micronized 5–100	3.6	4
Sulforon 5–100	6.6	45
Magnetic-Spray 5–100	8.3	23
Sulcoloid (1936) 5–100	14.2	170 ±
Nontreated	.....	200 ±
Series 2		
Micronized 7–100	3.6	4
Mike 7–100	5.5	21
Sulforon 7–100	6.6	19
Magnetic-Spray 7–100	8.3	3
Sulfix 7–100	8.8	24
Microsulphur 7–100	10.3	60
Nontreated	.....	130 ±
Series 3		
Micronized 5–100	3.6	7
Micro-Spray 5–100	6.7	13
Sulforon 5–100	6.6	16
Magnetic-Spray 5–100	8.3	22
Sulfix 5–100	8.8	40
Apple Dritomic 5–100	12.2	42
Sulcoloid (1936) 5–100	14.2	130 ±
Nonsprayed	.....	300 ±
Series 4		
Koppers Dry 7–100	2.0	7
Micronized 7–100	3.6	23
Mike 7–100	5.5	42
Sulforon 7–100	6.6	32
Magnetic-Spray 7–100	8.3	49
Sulfix 7–100	8.8	61
Apple Dritomic 7–100	12.2	150 ±
Sulcoloid (1936) 7–100	14.2	200 ±
Nontreated	.....	180 ±
Series 5		
Micronized 5–100	3.6	10
Micronized 2–100	3.6	49
Kolospray 6–100	7.7	19
Kolofog 6–100	....[c]	1
Nontreated	.....	100 ±

[a] Surface average diameter.
[b] Inches of artificial rainfall on turntable.
[c] Particle size of Kolofog could not be satisfactorily determined due to the complexity of the material.

jars until ready for sulphur analyses. Two grams of the dried leaf sample were used for each determination.

The method of sulphur analyses is that of Emerson (4) as modified by White (18). This modified method has been found particularly suitable

for analyzing sulphur in spray residues. It should be noted that these results give the total sulphur on or in the leaf. It is not possible to distinguish between the sulphur that is no longer fungicidal and that that is still in the toxic form. However, it is sufficient for denoting the relative order of retention of the sulphur samples.

Results. Analyses of Rome Beauty leaves sprayed with the 3.5, 6.6, and 10.3 μ experimental sulphurs before and after successive rain periods showed particle size to be an important factor influencing tenacity (Table 8 and Fig. 2). In all instances, the original deposits decreased greatly with the first rain. No doubt the coarse sulphur particles, and those particles not in intimate contact with the leaves were removed with the first washing rain. After the first inch of rainfall, the rate of sulphur loss was much slower.

TABLE 7.—*Cedar-apple rust control of potted Medina apple trees sprayed with copper ammonium zeolite (Z–O) of three different average particle sizes*

Series and treatment	Particle size[a]	Per cent of particles 4 microns and under	Max. lesions per sq. in. of leaf surface with given amounts of artificial rain	
			0.5-in. wash[b]	1.0-in. wash[b]
Series 1, Medina				
Special Z–O 3–100	1.6	94.4	12	24
	10.0	5.4	47	69
	13.3	1.5	51	79
Nonsprayed	.....	.....	90	.....
Series 2, Wealthy				
Special Z–O 3–100	1.6	94.4	6	15
	10.0	5.4	27	76
	13.3	1.5	42	.....
Nonsprayed	.....	.....	150 ±	.....

[a] Surface average diameter.
[b] Inches of artificial rainfall on turnable.

It is distinctly evident that the sulphur of larger average particle size continues to be removed at a faster rate than that of smaller average size (Table 8 and Fig. 2). It is shown graphically that after 0.5-inch precipitation, there were retained 71, 68, and 61 per cent of the original deposits of the 3.5, 6.6, and 10.3 μ sulphurs, respectively; whereas, after 4.3 inches of rain, there were retained 53, 39, and 34 per cent in the same order.

Although these differences between the amounts of sulphur retained might seem small, unpublished data indicate that they are enough to make the difference between infection and control. They are believed to present a true picture of what takes place in normal spray practice.

It is worthwhile to note that the factor of particle size did not affect the amounts of the original deposits, a complicating factor often encountered in attempting to compare commercial brands of sulphur.

These analyses of fungicide-weathering from mature apple leaves in midsummer show a relatively slow loss of sulphur. Greenhouse studies and field

experience indicate that protection would be lost more rapidly during the spring growing season.

Spore Germination Studies

Methods. To determine whether particle size affects the toxicity of sulphur and copper fungicides, a series of spore germination experiments were

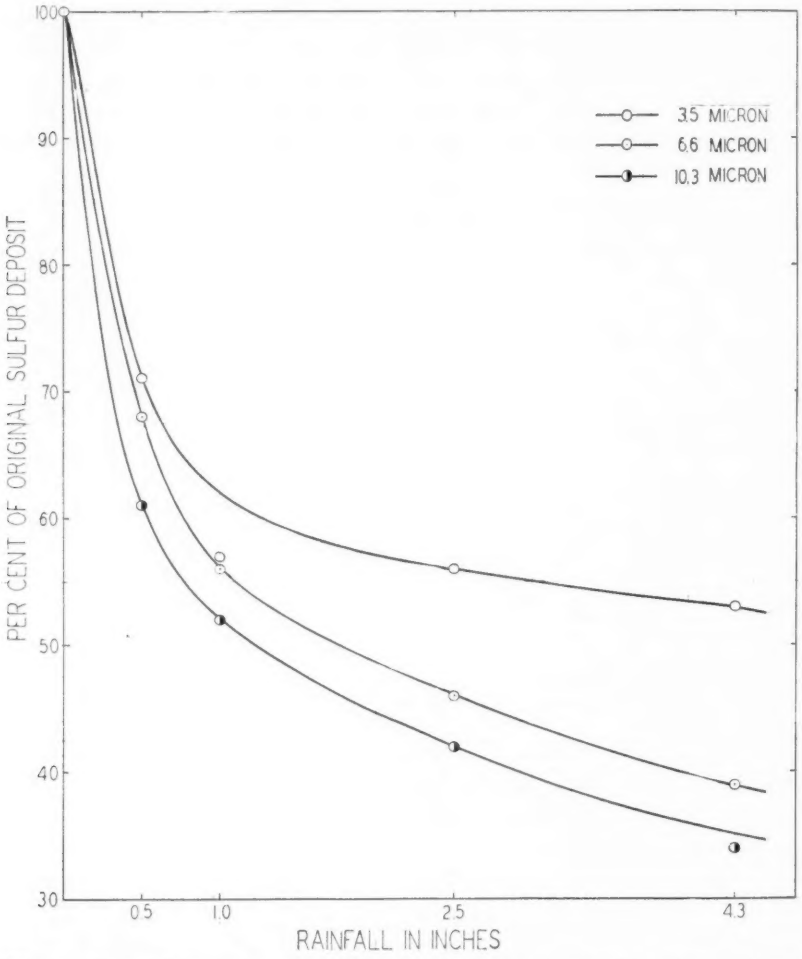

FIG. 2. Comparative retention of a special sulphur material of varying particle size to Rome Beauty foliage in the field.

made, using *Sclerotinia fructicola* (Wint.) Rehm as the test organism. The special wettable sulphur, in 3 particle sizes, was applied by a tower sprayer to glass slides coated with cellulose nitrate.[4] Copper zeolite, ground to 3 particle sizes, was sprayed on glass slides by means of a laboratory sprayer (14). *Sclerotinia fructicola* spores, from 7-day-old cultures, were suspended

[4] Hamilton, J. M., and G. L. Mack. A new vertical laboratory sprayer (in prep.).

in water containing synthetic media. (The synthetic media consisted of a stock solution of M/4 dextrose, M/5 KNO_3, M/20 KH_2PO_4, M/100 $MgSO_4$, and a trace of $FePO_4$.) This stock solution was diluted 800 times with redistilled water. The spore suspension was adjusted to a concentration of 60 spores per sq. mm. in a Fuchs-Rosenthal counting cell, and triplicate drops were placed on the slides with a standardized pipette. After incubation in moist chambers at a temperature favorable for germination, 100 spores were counted from each of 3 drops and averaged for the percentage of germination.

Results. The data show that the smaller the particle size, the greater the toxicity of the material (Tables 9 and 10). When the amount of fungicide deposited is plotted against the percentage of spores inhibited from germination by the fungicides, virtually straight parallel lines result for all materials.

TABLE 8.—*The comparative adhesiveness of fine, medium, and coarse sulphurs to foliage of Rome Beauty trees at successive intervals of rainfall*

Special sulphur treatments	Date of collection	Rainfall in inches	Mg. of sulphur per g. of dried leaf	Percentage sulphur retained
			Av. of 2 samples	
3.5 micron 4–100	7/20	0.0	2.74 ± .03	100
	7/27	1.02	1.80 ± .01	66
	8/12	3.92	1.31 ± .01	48
6.6 micron 4–100	7/20	0.0	2.71 ± .05	100
	7/27	1.02	1.37 ± .05	51
	8/12	3.92	1.22 ± .07	45
10.3 micron 4–100	7/20	0.0	2.79 ± .04	100
	7/27	1.02	1.17 ± .01	42
	8/12	3.92	0.82 ± .01	29

Photomicrographs of the inhibitory effect of sulphur materials of various particle sizes against the spores of *Sclerotinia fructicola* bear out the above assumption (Figs. 3 and 4). Under the conditions of these experiments, the relative length of the germ tubes is a better criterion of the inhibitory action of the sulphur than the percentage of the spores germinated. Commercial sulphur materials show the same trend in relation to particle size as the specially prepared samples in which particle size is the only variable factor (Fig. 4).

It might be assumed that differences in inhibition of spore growth were due to the fact that a given weight of small particles will cover the surfaces of the slides more thoroughly than the same weight of large particles and thus increase the chances of contact with the spores. However, an examination of the photomicrographs (Figs. 3 and 4) indicates that actual contact between spores and sulphur particles is no criterion of whether or not a given spore will germinate. While the mechanism of fungicidal action is not thoroughly understood, it seems logical to conclude that the finer particle materials were more toxic because of the larger surface area exposed to the water film in which the spores must germinate.

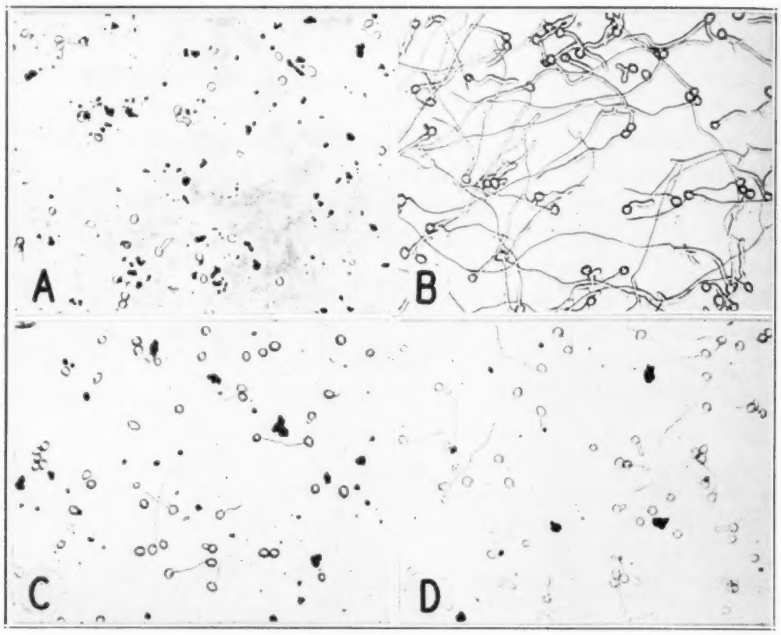

FIG. 3. The inhibition of spores of *Sclerotinia fructicola* grown *in vitro* in the presence of equivalent amounts of three grades of a special ground sulphur material varying only in particle size. Surface average diameter of the sulphur particles: A, 3.6 μ; C, 6.6 μ; D, 10.3 μ; B, control.

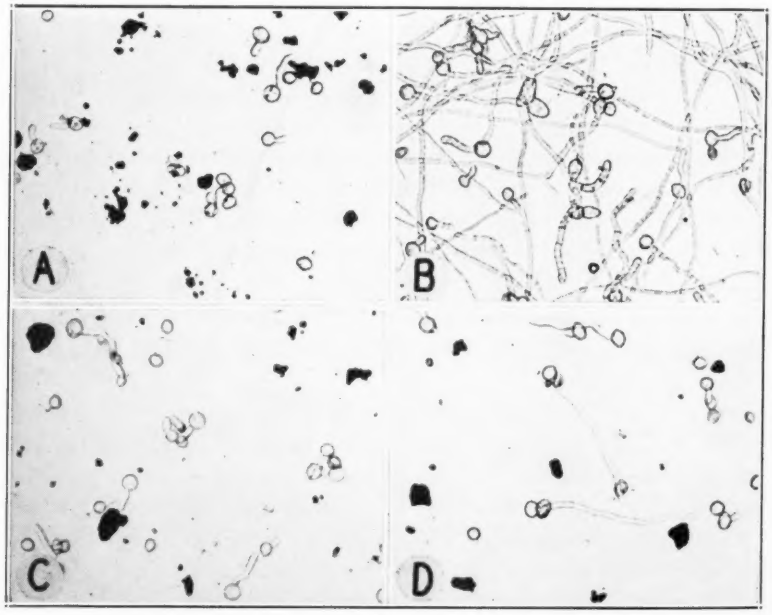

FIG. 4. The inhibition of spores of *Sclerotinia fructicola* grown *in vitro* in the presence of 7 micrograms per sq. cm. of commercial sulphur materials of various particle sizes. Surface average diameter: A, 3.6 μ; C, 6.6 μ; D, 8.8 μ; B, control.

TABLE 9.—*Toxicity of sulphur of three degrees of fineness to spores of Sclerotinia fructicola*

Particle size in microns	Spray time in seconds	Per cent of spore germination inhibited
		Average of 4 tests
3.5	30	41 ± 1
3.7		35 ± 1
6.6		21 ± 2
10.3		8 ± 1
3.5	60	48 ± 1
3.7		43 ± 3
6.6		31 ± 2
10.3		16 ± 1
3.5	90	54 ± 2
3.7		49 ± 2
6.6		39 ± 2
10.3		22 ± 2
3.5	120	58 ± 2
3.7		53 ± 2
6.6		46 ± 1
10.3		28 ± 1
Control	0	3 ± 0

SUMMARY

The fungicidal effectiveness of ground wettable sulphurs and an insoluble copper was found inversely proportional to the size of the particles. The correlation was established by experiments in the field, greenhouse, and laboratory.

A wettable sulphur and an insoluble copper, ground to 3 degrees of fineness to include the range of particle sizes found in most commercial mate-

TABLE 10.—*Toxicity of copper ammonium zeolite of three degrees of fineness to spores of Sclerotinia fructicola*

Particle size in microns	Spray time in seconds	Copper deposit micrograms per sq. cm.	Per cent of spore germination inhibited
			Average of 2 tests
1.6	3	0.7	27 ± 2
10.0			25 ± 1
13.3			2 ± 0
Commercial Z–O			2 ± 1
1.6	8	1.8	42 ± 1
10.0			39 ± 2
13.3			10 ± 2
Commercial Z–O			12 ± 2
1.6	13	2.9	60 ± 2
10.0			54 ± 2
13.3			33 ± 2
Commercial Z–O			28 ± 1
1.6	20	4.4	81 ± 1
10.0			73 ± 2
13.3			57 ± 2
Commercial Z–O			52 ± 2
Control	0	0.0	2 ± 1

rials, were tested against apple scab, *Venturia inaequalis* (Cke.) Wint., apple rust, *Gymnosporangium juniperi-virginianae* Schw., and brown rot, *Sclerotinia fructicola* (Wint.) Rehm. Similar experiments were conducted with commercial wettable sulphurs whose fungicidal properties were affected not only by particle size but also by the presence of various adjuvants, and by different methods of manufacture. Sulphur materials prepared by the Grinrod and flotation processes were relatively less adhesive than ground sulphur of equal particle size, provided no material was added to aid retention.

The particle-size measurements were checked by 4 different methods and fair agreement was obtained. The variation in results obtained by different methods is discussed and it is concluded that the Andreasen sedimentation method and the air-permeation method are the most reliable procedures.

Photomicrographs of the particles in representative materials further confirmed the results of the particle-size determinations. These pictures emphasize the fact that for a given weight of material there are many more particles per unit area for a fine powder than for a coarse one.

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REACTION OF RESISTANT TOBACCOS TO CERTAIN STRAINS OF NICOTIANA VIRUS 1 AND OTHER VIRUSES[1]

H. H. McKinney[2]

(Accepted for publication November 8, 1942)

INTRODUCTION

Data presented in another paper (13) show that certain collections of tobacco from Colombia, which are exceedingly unfavorable hosts for Nicotiana virus 1, rarely manifest gross signs of disease when infected experimentally. However, when these highly resistant plants are infected with certain yellow-type mutants from Nicotiana virus 1, symptoms may or may not result, depending on the conditions of culture. Tests were carried out with several virus mutants isolated by the writer, with several varieties or strains of Nicotiana virus 1, collected in the field by the writer and others, and with several viruses outside the common-mosaic group.[3]

MATERIALS AND METHODS

The plant materials employed in the research herein reported were described in another paper (13). They comprised 3 tobaccos, *i.e.*, 1. Wisconsin-Havana Seed, very susceptible; 2. Ambalema, resistant; 3. T.I. 448A, very resistant; and 4. *Nicotiana glauca* L. (8).

Most of the assays were made on the primary leaves of *Phaseolus vulgaris* L., var. Scotia, in accordance with the methods outlined previously (13), but other testing species were used for certain viruses as indicated throughout the paper. In certain of the assays, 600-grain carborundum powder was dusted on the bean leaves before wiping with the extracts.

Those viruses used were: Nicotiana virus 1 and seven distinct mutants isolated from Nicotiana virus 1. Although none of these mutants can be regarded as slow-moving in Wisconsin-Havana Seed tobacco, they constitute a graded series for the degree of chlorosis incited, beginning with the mild-green mosaic mutant *AMG* (12), and ending with the severe yellow-mosaic mutant *BSY* (12).[4] "White" mosaic virus[5] collected in the field by W. D.

[1] Studies supported in part by funds provided under Bankhead-Jones Project S.R.F. 2–17, Agricultural Research Administration, U. S. Department of Agriculture. These studies were cooperative between the Bureau of Plant Industry and the Bureau of Agricultural Chemistry and Engineering.

[2] Senior Pathologist, Division of Cereal Crops and Diseases. Acknowledgment is due Matthew Koerner for assistance in conducting the tests; W. D. Valleau, E. E. Clayton, S. P. Doolittle, E. S. Schultz, and T. P. Dykstra for supplying virus or other materials.

[3] Smith's (18) system of nomenclature is used for those viruses he has listed.

[4] The term yellow mosaic as used by the writer has always been based on the pigment coloration in dark tobacco. When mutant *BSY* was isolated in 1926, the writer then attempted, without success, to isolate a virus that might induce a bleached or white mottling. Since then he has tried several times to obtain such an isolation, but without success. When the writer's yellow-mosaic *BSY* was studied on light-green tobaccos of the Burley type, white mosaic resulted. The normal plastid pigment content of these tobaccos is so low in comparison with that in the dark tobaccos, that the yellow pigments frequently reach their end points in a large percentage of the mottled tissue. Since all of the research on isolated yellow-mosaic mutants has been done with the dark green tobaccos, and since

Valleau (19); Plantago-mosaic virus collected by the writer, and similar to *Marmor tabaci* var. *plantaginis* described by Holmes (5); Type *B* yellow-mosaic virus (9); Nicotiana virus 6 (12); Type *C* yellow-mosaic virus (9); Solanum virus 2, veinbanding-mosaic source; Solanum virus 2, "Y"-mosaic source; Cucumis virus 1, cucumber-mosaic source; Cucumis virus 1, celery-mosaic source, and Nicotiana virus 12 (tobacco ring spot).

The experiments were conducted in a greenhouse and in controlled-temperature chambers. All tests were made in daylight. The methods of culture were the same as those previously described (13).

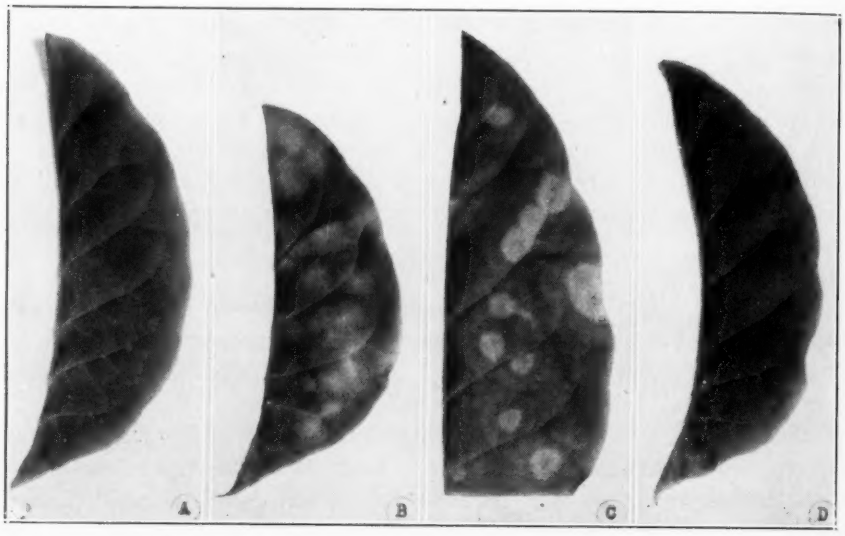

FIG. 1. Portions of leaves of tobacco T.I. 448A illustrating the influence of age of plants on the expression of symptoms when yellow-mosaic mutant *BSY* is used for the inoculum. A. Healthy control. B. Wiped half-leaf of very young plant showing yellow primary spots. C. Half-leaf from same plant as (B) and above it showing yellow spots from systemic virus. D. Half-leaf from a plant 3 weeks older but inoculated from the same virus extract and at the same time as the very young plant. This leaf contained virus.

EXPERIMENTAL RESULTS

Reaction of T.I. 448A to Some of the Mutants from Nicotiana Virus 1

Several preliminary tests were carried out in the greenhouse with plants of T.I. 448A, ranging from 40 to 80 days old from date of seeding. It was found that the mild-green mosaic mutant *AMG* induced no symptoms under any conditions, though virus was isolated from the inoculated plants. In young plants 40 days old, all 6 of the yellow-mosaic mutants that were tested

green dark tobaccos have been for so long used and by so many workers, it is evident that if light tobaccos are employed this fact should be so stated by the experimenter. Otherwise, there will be resultant confusion.

[5] The term "virus" as applied here and elsewhere in this paper, signifies no virus of specific rank, but merely an infectious entity. This usage compares with that of such terms as pathogen, parasite, infectious agent, fungus, bacterium, etc. Throughout the paper this usage has been made clear by the context.

induced primary and secondary yellow spots, but in plants 49 days old, and older, irregularities became more evident; 3 of the yellow-mosaic mutants failed to induce secondary symptoms; primary symptoms were faint and sometimes absent. Figures 1 and 2 illustrate resistance coincident with age and genotype.

These results made it very clear that some of the mutants were less infective than others and that other factors were operating. It appeared that

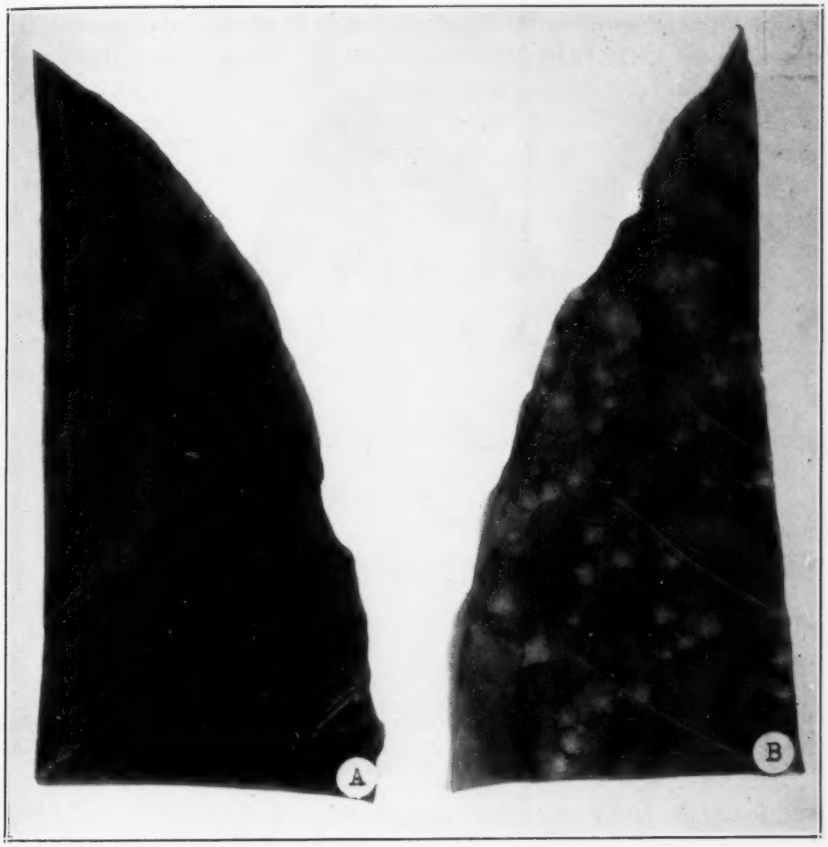

FIG. 2. Portions of leaves from large tobacco plants wiped with yellow-mosaic mutant *BSY*. A, T.I. 448A; B, Wisconsin-Havana Seed.

age of plant and temperature are important. As mutant *BSY* (12) seemed to be less erratic than some of the other yellow-mosaic mutants, it was used in much of the subsequent work.

Effect of Age of Plants. In young plants inoculated with *BSY* by the leaf-wipe method, and cultured near 22° C., secondary chlorotic spots developed on 7 to 17 leaves above the inoculated leaves. The number of chlorotic spots and their intensity in yellow color decreased on the leaves progressively up the plants. Secondary chlorotic spots frequently developed a

concentric pattern (Fig. 1 C). In comparison with the highly susceptible control plants, the primary chlorotic spots usually appeared from 1 to 3 days later on the wiped leaves of T.I. 448A.

The first signs of secondary chlorotic spots usually appeared at the tip and margins of leaves that were $\frac{3}{4}$ to $1\frac{1}{4}$ inches long when the plant was inoculated. As these leaves enlarged, new spots appeared progressively towards the petioles; and there was considerable spread of chlorosis from the original centers, especially near the apical margins of the leaf. Several subsequent leaves developed chlorotic spots in the same manner; but, since the number of spots per leaf became progressively less in subsequent leaves,

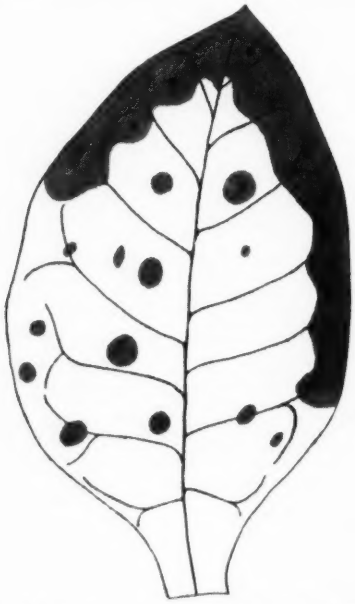

FIG. 3. Diagram of leaf from young plant of T.I. 448A (third above the inoculated leaf); virus of yellow mutant *BSY* used; the darkest spots denote the initial, discrete yellow spots which appeared; the gray spots and areas denote spots which appeared later and also the spread of chlorosis from yellow centers.

the sequence of their appearance from the tip to the base of the leaf became less striking. By far the majority of chlorotic spots was centered in the mesophyll traversed by the smallest veins. Very few spots had their centers in the veins, and most of these appeared in the marginal region where the veins are small (Fig. 3).

Unlike the mosaic mottling of Wisconsin-Havana Seed tobacco, the chlorotic spots did not appear on the very small central leaves at any stage of the plant's development.

Eight plants of T.I. 448A, 66 days from seedling, were inoculated with fresh virus extract of *BSY*. Four of these plants were inoculated by means of needle punctures in the leaf axils near the growing tip and four were inoculated by wiping nondiluted virus extract on 3 leaves.

No symptoms appeared in and no virus was recovered from the plants inoculated by means of the needle. Local chlorotic spots appeared on all the leaves in the wiped series, one plant developed secondary symptoms on 5 leaves and 3 plants failed to show secondary symptoms.

A plant of T.I. 448A and a plant of Wisconsin-Havana Seed, each about 80 days old, were inoculated by wiping the leaves with nondiluted fresh extract of *BSY*. No local chlorotic spots appeared on the wiped leaves of T.I. 448A, but from 50 to 400 spots appeared on the wiped leaves of Wisconsin-Havana Seed (Fig. 2). When the primary symptoms appeared on Wisconsin-Havana Seed tobacco, one-half of each of 11 leaves from each of the varieties was assayed. The leaf samples were thoroughly pulped in an equivalent amount of buffered solution by weight and the resulting extracts, without further dilution, were wiped on Scotia bean leaves. The extracts from the leaves of Wisconsin-Havana Seed induced from 212 to over 500 lesions per bean plant. Four of the leaf samples from T.I. 448A failed to induce lesions, 6 failed to induce more than a trace, which might represent residual virus, and 1 leaf induced an average of only 12 lesions per bean plant. Twelve days later the remaining halves of leaves on the T.I. 448A plant were assayed. The highest level of activity was in a sample that induced 41 lesions per bean plant.

When the leaves of large plants of T.I. 448A were dusted with 600-grain carborundum powder and wiped with strain *BSY*, local chlorotic spots appeared in large numbers. In one test, the effect of carborundum was determined on half leaves. The number of local spots on the halves receiving no carborundum ranged from 0 to 10, and on those receiving the powder, the number ranged from 172 to over 300. The latter figure could not be determined accurately as the spots were too numerous.

Assays indicate that mutant *BSY* is not present in the upper portion of mature infected plants of T.I. 448A. This virus-free zone seems just as extensive, if not more so, when *BSY* is applied, as is the case when Nicotiana virus 1 is used (13).

In young plants of T.I. 448A, mutant *BSY* has not been isolated from the growing points and smallest tip leaves when the typical secondary symptoms obtain in the large leaves. In one test, a group of plants showing typical symptoms was used for making virus assays on extracts from the young symptomless leaves. These leaves were divided into 3 groups as follows: (1) growing points and tip leaves 15 mm. long and shorter; (2) leaves next below (1) and 22 to 35 mm. long; and (3) leaves next below (2) and 50 to 65 mm. long. These samples were finely pulped in 4 parts of buffered solution and wiped on bean leaves previously dusted with carborundum powder. No necrotic lesions were induced by any of the extracts. In similar tests, traces of virus have been detected occasionally in leaves 50 to 65 mm. long, but virus is rarely detectable in leaves 30 to 40 mm. long.

Since carborundum powder greatly increases the number of primary infection sites in T.I. 448A, it was thought that typical mosaic mottling

might occur if concentrated virus extracts were wiped on all the leaves of plants dusted with carborundum powder. However, in spite of the myriads of primary sites that developed, there was not a single case of mosaic mottling in 60 T.I. 448A plants so treated.

In susceptible tobacco such as Turkish, variety Samsun, and Wisconsin-Havana Seed, *BSY* has been detected without fail in the growing points and smallest leaves from plants showing the typical yellow-mosaic symptoms, even when the samples were diluted to 10^{-4} with buffered solution. In such a test, run concurrently with that cited in the preceding paragraph, the growing points and small mottled leaves 6 mm. long contained sufficient virus to induce 124 lesions per bean plant, and mottled leaves, 35 to 40 mm. long, from the same plants, induced 400 lesions per bean plant.

Effect of Temperature. Experimental consideration was given to the effect of temperature on infection and on expression of symptoms in T.I. 448A and Ambalema plants inoculated with Nicotiana virus 1 and its mutant *BSY*. Plants 64 days old were used. Counting tip leaves $\frac{1}{2}$ inch long, the plants bore 8 to 9 leaves each. Three well-developed green leaves were wiped with nondiluted fresh virus extracts. Three plants of each combination of virus and variety were placed in each of 3 temperature chambers controlled near 22.5° C., 28.5° C., and 33.5° C., respectively. Winter daylight was the only source of illumination.

The results are given in table 1, and samples of the leaves wiped with strain *BSY* are illustrated in figure 4. Leaves wiped with Nicotiana virus 1 are not illustrated, as they developed no symptoms. The necrotic lesions induced by *BSY* on Ambalema at 28.5° C. (Fig. 4, E) were very small. They started as minute brown points resembling those at 22.5° C. (Fig. 4, D); but, unlike the latter, they made more progress, and developed light-green to yellow margins. On the leaves of T.I. 448A, no necrotic lesions appeared at any of the temperatures, but diffuse yellow zones did develop at 28.5° C. and 33.5° C. (Fig. 4, A, B, C).

It is of interest to note that, in Ambalema at 22.5° C. and 28.5° C., no secondary cholorotic spots appeared, and assays failed to reveal systemic virus in these plants. At these same temperatures, some of the plants of T.I. 448A manifested secondary chlorotic spots. The tendency for Ambalema to produce only local necrotic lesions at the lower temperatures and to develop secondary symptoms at the highest temperature is similar to, although not identical with, the situation obtaining in *Nicotiana sylvestris* L. when inoculated with *BSY* (17), designated in that paper as yellow-mosaic virus (type *A*).

It will be observed in table 1, that Nicotiana virus 1 induced a few secondary light-green spots and oak-leaf patterns in both T.I. 448A and Ambalema cultured at 33.5° C. These symptoms involved but 1 to 3 leaves on each plant. The oak-leaf patterns consisted of rather faint outlines having the general form of the patterns induced on old leaves of Wisconsin-Havana Seed tobacco infected with some of the yellow mosaics. The writer illustrated this reaction in an early paper (8, Fig. 8).

TABLE 1.—*Temperature in relation to the expression of symptoms in young plants of T.I. 448A and Ambalema tobacco. Tests conducted during December and January*

Virus used	Symptoms at temperatures and on varieties indicated					
	22.5° C.		28.5° C.		33.5° C.	
	T.I. 448A	Ambalema	T.I. 448A	Ambalema	T.I. 448A	Ambalema
Nicotiana virus 1	No primary symptoms	No primary symptoms	No primary symptoms	No primary symptoms	Very few primary light-green spots	Very few primary light-green spots
	No secondary symptoms	No secondary symptoms	No secondary symptoms	No secondary symptoms	Very few secondary light-green spots and "oak-leaf" patterns on a few leaves of 3 plants	Very few secondary light-green spots and "oak-leaf" patterns on a few leaves of 3 plants
Yellow-mosaic mutant *BSY*	No primary symptoms	Many small brown pin-point necrotic spots on all wiped leaves	Light-green to yellow spots on all wiped leaves	Many small necrotic lesions and pin-point spots on all wiped leaves. Light-green margins around lesions	Many large yellow spots on all wiped leaves	Many large yellow spots on all wiped leaves
	Secondary chlorotic spots on 11 leaves on each of 2 plants; 1 plant free	No secondary symptoms	Secondary chlorotic spots on 8 leaves of 1 plant; 2 plants free	No secondary symptoms	Secondary yellow chlorosis, which gradually spread over most of the surface of 3 leaves on 1 plant. Two plants free	Secondary yellow chlorosis, which gradually spread over most of the surface of 3 leaves on 3 plants

Mutant *BSY*, at 33.5° C. did not induce primary chlorotic spots of a discrete type in either T.I. 448A or Ambalema. Instead, diffuse yellow

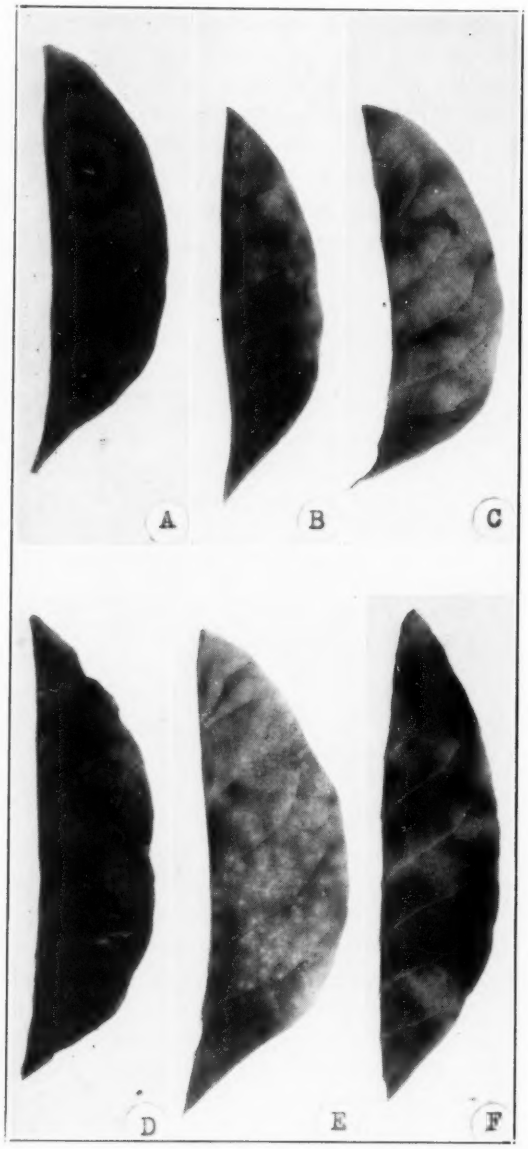

FIG. 4. Portions of leaves illustrating the influence of temperature on primary symptoms in young plants. A, B, and C, from T.I. 448A, incubated at 22.5° C., 28.5° C., and 33.5° C., respectively; D, E, and F, from Ambalema incubated at 22.5° C., 28.5° C., and 33.5° C., respectively.

patches appeared near the bases of the leaves and along the midrib and large veins. This chlorosis took on the form of the oak-leaf pattern, but this

effect was obscured as chlorosis finally involved most of the surface of a leaf. This reaction occurred in 3 leaves on each plant. It was impossible to continue this test through the entire vegetative period.

In another test, with mutant *BSY*, plants of T.I. 448A, but younger than those referred to in table 1, developed chlorotic spots on wiped leaves at temperatures near 23° C. Younger plants also have shown secondary symptoms in a more severe form at temperatures near 28.5° C. than was manifested at this temperature in the above test. However, even very young plants of T.I. 448A sometimes fail to manifest secondary symptoms. In this test 21 small plants of T.I. 448A were inoculated with concentrated virus extract of *BSY;* 3 to 4 leaves on each plant were wiped; virus also was introduced into the stem tip of each plant by means of a needle. The culture temperature was near 23° C. Secondary chlorotic spots appeared in 10 leaves on 4 plants; in 8 to 9 leaves on 9 plants; in 6 to 7 leaves on 6 plants; and in 2 leaves on 1 plant. In one plant there were no secondary symptoms. There is no evidence that the plants manifesting these different reactions and nonreaction represent distinct genotypes. The variability is explained on the basis that T.I. 448A is a poor suscept, in which case disease expression is subject to relatively slight differences in conditions that would not be reflected in highly susceptible plants.

Localization of Nicotiana Virus 1 and Its Mutant *BSY* in T.I. 448A

Tests with Mutant BSY.—Preliminary data and observataions suggested that the major increase of virus in T.I. 448A is confined to relatively few scattered zones, and as previously reported (10), this was found to be the case.

One of the first leaves of T.I. 448A to manifest severe secondary chlorotic spotting from mutant *BSY* was removed, and all spots were carefully located on a sketch illustrated in figure 3. Assays were made from (1) the yellow tissue, (2) the normal green tissue, (3) the large lateral veins, and (4) the midrib. All green tissue, lateral veins, and midrib tissue adjoining the yellowish-green margins of the yellow tissue were discarded. From 2 to 3 mm. of normal green tissue was left attached to and assayed with the lateral-vein tissue. The tissues were pulped thoroughly in 3 parts of buffer by weight, and the extracts were wiped on the primary leaves of Scotia bean plants. The lesion counts for the 4 tissues are listed in table 2.

TABLE 2.—*Virus concentration in yellow tissue and in green tissue of leaf of T.I. 448A tobacco infected with mutant BSY as shown by assays on Scotia bean plants. Diagram of leaf shown in figure 3*

Tissue tested	Lesions per plant
Yellow zones	701.3
Green lamina, no signs of chlorosis	48.0
Lateral veins	46.7
Mid-veins	0.3

These data indicate that the virus is more abundant in, but not confined to the yellow zones. Since the counts for the lateral veins and for the green tissue are so close, it seems reasonable to conclude that the bulk of the virus in the vein sample was in the attached green tissue. The low count from the midrib seems to indicate very little virus in the large vascular channels.

Another leaf bearing a single yellow spot, was cut into sections and each section thoroughly pulped in 0.5 cc. of buffered solution and assayed on bean leaves. The number of lesions per bean plant induced by each section of leaf tissue is given in figure 5. It will be observed that all detectable virus was located in and around the yellow spot, and that part of the normal green tissue near the yellow spot showed slightly greater virus activity than the yellow spot.

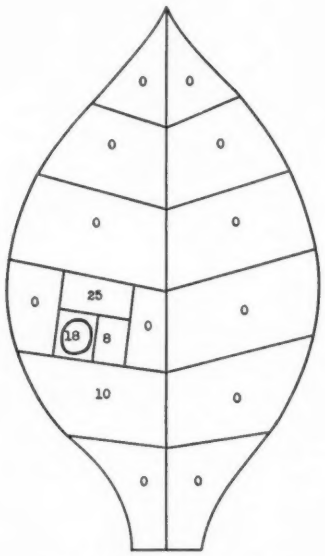

FIG. 5. Diagram of young leaf from plant of T.I. 448A, second above leaf inoculated with virus of yellow mutant *BSY*. Circle denotes a yellow spot; all sections of leaf outlined were assayed for virus; numerals denote number of lesions per bean plant.

A plant of T.I. 448A infected with mutant *BSY* exhibited chlorotic spots on 4 consecutive leaves. The 8 leaves above these appeared normal in every way. The 4 spotted leaves and 4 apparently normal ones immediately above were assayed separately, each on 4 bean plants. Extracts from entire leaves were expressed from fine-ground tissue without dilution. The results in table 3 show that detectable virus is not confined to leaves exhibiting the yellow spots.

Test with Nicotiana Virus 1.—A test was carried out to determine the systemic distribution of Nicotiana virus 1 in a leaf of T.I. 448A. Part of a leaf 22 cm. long was removed from an infected plant. Preliminary assays on parts of this leaf had shown the presence of virus, but there were no visible symptoms. The portion used in this test was cut into 19 sections con-

forming to the main vascular channels and leaf lamina (Fig. 6). Each section was finely ground in 3 parts of buffered solution, and the extracts were wiped on bean leaves. The numerals in the sections in figure 6 indicate the number of necrotic lesions per bean plant induced by the extracts from the sections. It will be observed that nearly all of the detectable virus occurred in 3 adjacent sections of laminal tissue.

The data cited in this chapter show that Nicotiana virus 1 and mutant *BSY* are localized to a very high degree in T.I. 448A tobacco. *BSY* virus tends to be most concentrated in the chlorotic zones, but it is not confined to them. This observation is at variance with the results reported by Valleau and Diachun (19). They succeeded in isolating virus only from the yellow zones in resistant tobacco leaves infected with their "white"-mosaic virus.

TABLE 3.—*Tests for presence of mutant BSY in spotted and adjacent nonspotted leaves of T.I. 448A tobacco, as shown by assays on Scotia bean plants. Extracts not diluted*

Leaf No.[a]	Yellow spots on leaf	Lesions per plant
	Number	*Number*
10	12	361
11	6	127
12	20	216
13	5	57
14	0	40
15	0	83
16	0	77
17	0	12

[a] Counts begin at base of stem. Leaf 21 was 2.5 cm. long, leaf 17 was 28 cm. long, and had not quite reached its full size.

The results of similar tests on leaves of T.I. 448A infected with Valleau's "white"-mosaic virus are cited later.

Observations Relating to Typical Mosaic Mottling in Wisconsin-Havana Seed

The results obtained with mutant *BSY* in T.I. 448A suggested a series of observations on mosaic-mottled leaves of Wisconsin-Havana Seed tobacco infected with *BSY* and supplementing those reported in 1929 (8). At that time the writer called attention to and illustrated the unusual symptoms that develop in the leaves of Wisconsin-Havana Seed tobacco when *BSY* virus invades well-developed, mature leaves. Reference to figure 8 in that paper (8) will show the characteristic "oak-leaf" pattern. These early observations made it clearly evident that the types of chlorotic symptoms induced in leaves are determined to a large extent by the stage of development of the leaf at the time of invasion.

Leaves of Wisconsin-Havana Seed tobacco with typical yellow-mosaic mottling were observed from the time they were 10 to 15 cm. long until they were mature. Normal green zones were carefully observed and it was

found that many of them remained normal green only a short time. In many cases yellow spots appeared, which resembled the secondary yellow spots that occurred in T.I. 448A tobacco.

Microscopic studies were carried out on small pieces of the various types of spotted, and mottled tissues and the normal green tissues cut from infected leaves of Wisconsin-Havana Seed tobacco. These pieces of tissue were dropped into absolute alcohol for a few minutes, then transferred to xylol for a like period. Partly cleared, they were then mounted in xylol and viewed through a compound microscope. With a magnification of 100×, it was possible to see through the epidermal cells and observe and count the palisade cells in both green and yellow areas.

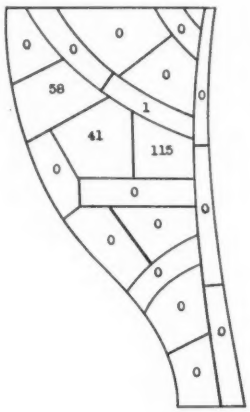

FIG. 6. Diagram of part of leaf from a plant of T.I. 448A infected with Nicotiana virus 1; no manifestation of symptoms on leaf; all sections outlined were assayed separately for virus; numerals denote number of lesions per bean plant.

These observations showed that (1) mottled leaves frequently contain normal green patches in which no yellow cells are detectable under the compound microscope; (2) patches that at first are normal green to the eye, in many cases develop a light or faded green; (3) these faded areas consist of small islands of palisade cells containing chlorophyll surrounded by yellow palisade cells; (4) the small green islands range from 1 to 20 or more palisade cells; (5) the faded green areas frequently, but not always, become completely yellow; and (6) this secondary yellowing in the normal green patches sometimes involves all palisade cells from the beginning of the process, resembling the progress of the secondary yellowing in perennial pepper leaves that have the local lesions of mutant *BSY* (14).

It seems reasonably evident from the observations cited that the occurrence of the typical mosaic patterns in foliage is dependent on virus entering the very small leaves, and that the mottling is modified by secondary chlorosis as the leaf develops.

Inoculations with Miscellaneous Field Varieties of Nicotiana Virus 1 and Other Viruses

"White"-mosaic virus. This virus was supplied by W. D. Valleau. It is very similar to, but not identical with mutant *BSY*. In dark tobacco it induced severe yellow mosaic and was almost indistinguishable from *BSY* mosaic. In *Nicotiana glauca* it was isolated from top leaves in 80 per cent of the mature plants; it induced stem mottling and chlorotic spots in some of the leaves throughout the life of these plants. Mutant *BSY* was not completely systemic in any of the plants, and induced very few or no secondary chlorotic spots (9).

Mutant *BSY* and "white"-mosaic virus were each tested against Nicotiana virus 1 in Wisconsin-Havana Seed tobacco to determine the relative interference[6] or antagonism. The methods applied were the same as those reported for use with systemic viruses (12). It was found that Nicotiana virus 1 supplanted both of the other viruses, and that *BSY* virus was supplanted more quickly than the "white"-mosaic virus.

In two tests with T.I. 448A, the "white"-mosaic virus induced secondary light-green and yellow spots in 8 to 10 leaves per plant, with a maximum of 250 spots per leaf, whereas *BSY* virus induced similar spots on 4 to 5 leaves per plant, with a maximum of 25 spots per leaf.

Three leaves, each from a separate plant of T.I. 448A, were chosen for study. Leaf 1 had 22, leaf 2 had 77, and leaf 3 had 271 systemic yellow spots ("white" mosaic). There was enough normal-green tissue in each leaf to provide samples well removed from yellow spots. Tissue samples consisted of round discs 19 mm. in diameter. One disc was removed from an area containing 10 yellow spots; 12 discs, 4 from each leaf, were taken from normal-green zones flanked by scattered yellow spots at distances ranging from 5 to 20 mm. from the margins of the discs. Each disc was pulped in 3 cc. of buffered solution, and the resulting fluid was wiped on the two primary leaves of 3 bean plants.

The 4 normal-green discs from leaf 1 induced 0, 0, 1, and 1 lesions, respectively, on the 6 bean leaves; those from leaf 2 induced 800, 700, 300, and 1 lesions, respectively, per bean plant; and those from leaf 3 induced 90, 35, 0, and 0 lesions, respectively, per bean plant. The disc from the spotted tissue induced 1400 lesions per bean plant. The high counts were based on close estimates.

Fourteen days following the above assays, a few light-green and yellow spots appeared in some of the areas that were normal green at the time the sample discs were removed. It is not surprising, therefore, that virus was isolated from some of the normal-green tissue.

[6] In recent years it has been found that certain viruses attacking the mammals, exhibit antagonism. The phenomenon occurs between certain yellow-fever viruses and the Rift-Valley-fever virus (3), between some of the viruses of the poliomyelitis group (6), and between the virus of canine distemper and experimental poliomyelitis virus (2). Some virologists have regarded the phenomenon as a form of immunity in mammals, but the present trend is to refer to it as a "sparing effect" (2) and "interference" (3, 6), and to regard the phenomenon as being quite distinct from acquired immunity. This position is in line with the position taken by the writer (11) regarding the phenomenon in plants.

In another test with T.I. 448A, "white"-mosaic virus induced yellow spots in 4 consecutive leaves above the inoculated leaf. In the next 4 consecutive leaves, faintly chlorotic spots appeared that were detectable only in transmitted light, whereas, no signs of disease appeared in any of the well-developed leaves above nor in the tip leaves. Nondiluted extracts from each of these symptom-free leaves and from the tip cluster of small leaves were assayed on bean leaves. Proceeding in order up the plant, these extracts induced local lesions at the rate of 800, 30, 400, 2, 0, and 0 lesions per bean plant.

Trials with tobacco genotypes that carry the necrotic-lesion factor and trials with *Nicotiana sylvestris* Spegaz. and Comes, have shown these types to be inferior test plants for detecting traces of necrotic-lesion-inducing viruses. *Phaseolus vulgaris* L., when grown near 33.3° C. after inoculation, and *Nicotiana glutinosa* L., appear to be among the best known test plants for determining traces of the viruses under discussion. The fact that Valleau and Diachun (19) used a tobacco for their testing suggests one possible explanation for their failure to detect traces of virus in their normal-green tissue.

"White"-mosaic virus, like *BSY* virus, was not isolated from the growing tips of the stems and small leaves of infected plants of T.I. 448A. A typical test is cited. The tissue extracts were diluted in 5 parts of buffered solution and wiped on bean leaves dusted with carborundum powder. The extract from the growing points and smallest leaves and the extract from leaves 2.5 to 5 mm. long induced no lesions, whereas the extract from leaves 70 to 90 mm. long induced 350 lesions per bean plant. There were no chlorotic spots discernible on any of the tobacco leaves tested.

Type B Yellow-mosaic Virus. This virus was isolated from *Nicotiana glauca* L., growing in the Canary Islands (8). It is very similar to mutant *BSY*, but, unlike *BSY*, it induces severe yellow mosaic in *Nicotiana glauca* (9). In T.I. 448A tobacco, however, the two viruses induce symptoms that are practically indistinguishable.

Plantago-mosaic Virus. This virus was collected in 1941 on *Plantago major* L. at Arlington Farm, Virginia. On this species the disease is distinctly a ring spot. It was found in 3 separate locations, 2 of which were within a few feet of the entrances to 2 separate greenhouses; the other was close to a laboratory entrance. Common mosaic of tobacco had long been studied in these greenhouses and in the laboratory. Search for this disease on *P. major* and *P. lanceolata* L. in nearby Virginia, Maryland, and the District of Columbia has met with failure. Experiments have shown that infected leaves of *P. major* frequently show no signs of disease. This virus may be a strain of *Nicotiana virus* 1; it is similar to the virus collected by Holmes (5) on *P. major* and *P. lanceolata*, but, unlike his virus, it rarely induces local lesions, ring spots, or oak-leaf patterns on Turkish tobacco, var. Samsun.

In tobacco T.I. 448A, the virus induced no symptoms. Systemic virus was recovered from only one of the 30 inoculated plants, and this plant was one of a series of five inoculated by the carborundum-wipe method. The tests conducted thus far indicate that T.I. 448A tobacco manifests an unusually high degree of resistance against this virus.

Nicotiana Virus 6. This virus induced no local or secondary symptoms in T.I. 448A and only occasional, faint, light-green spots in Ambalema. In both genotypes, the virus seems to synthesize and progress much like Nicotiana virus 1.

Type C Yellow-mosaic Virus. This virus and Nicotiana virus 6 seem to belong to a closely related group occurring in *Nicotiana glauca* growing in the Canary Islands (9). In T.I. 448A no local or secondary symptoms occurred, and the virus made very slight progress in the few plants that have been studied.

Solanum Virus 2. Virus from veinbanding mosaic and virus from the "Y" mosaic induced typical symptoms in T.I. 448A. This tobacco is regarded as highly susceptible to both of these mosaics.

Cucumis Virus 1. Virus from the milkweed-cucumber source induced mosaic without necrosis in T.I. 448A; but virus from the celery-mosaic source induced local chlorotic spots and compound necrotic lesions and systemic necrosis in stems and young leaves.

Nicotiana Virus 12 (tobacco ring spot). T.I. 448A is very susceptible to the acute symptoms, and manifests the low-temperature chlorosis and mosaic mottling associated with the chronic phase (11) of this disease.

DISCUSSION AND CONCLUSIONS

When viruses of the yellow-mosaic mutants were used to inoculate tobacco T.I. 448A, the secondary chlorotic spots rarely appeared in the new leaves until they had attained about $\frac{1}{4}$ to $\frac{1}{2}$ of their growth. Traces of virus, however, were detected occasionally in tip leaves 5 to 6 cm. long. The virus either does not enter or increase perceptibly in the very young leaves, which probably explains the absence of true mosaic mottling in T.I. 448A. The first signs of secondary chlorotic spots usually appear at or near the tip of the leaf. Spotting tends to progress down the leaf as it grows. The spread of chlorosis from the initial spots tends to be greatest in the leaf margins and tip. Thus it appears that this spread is not correlated with the rate of cell division, as division is less rapid toward the tip than it is in the lower margins (1).

The evidence available makes it rather conclusive that the secondary chlorotic spots in T.I. 448A and Ambalema result from the destruction of chlorophyll rather than from the prevention of chlorophyll synthesis. Products of a deranged metabolism may be the direct cause of the destruction of chlorophyll in these tobaccos, but good circumstantial evidence of this cannot be obtained so readily as in perennial pepper, *Capsicum frutescens* L. (14).

In the highly susceptible genotypes, such as Wisconsin-Havana Seed tobacco, it appears that the amount of virus in the very small leaves, although less than in leaves at a more advanced stage, is sufficient to induce the necessary reactions that prevent or reduce the synthesis of chlorophyll in certain groups of parenchyma cells. It may be that these cell groups are more susceptible than those in which the normal quota of chlorophyll is produced. However, from the observations cited for T.I. 448A and Wisconsin-Havana Seed tobaccos, it appears more likely that the initial quantity of virus entering the youngest leaves in a highly susceptible tobacco may be too small to insure all parenchyma cells receiving their quota of virus before the chlorophyll is synthesized, and that those cells receiving virus after the appearance of chlorophyll do not become chlorotic for a time at least, depending on the virus used. Thus, the classical mosaic pattern results.

With common mosaic (Nicotiana virus 1), the classical mosaic pattern in dark-green tobaccos tends to change very little as the leaves develop, whereas, with yellow mosaics, the mosaic pattern tends to be less permanent, becoming modified because of the advance of delayed chlorosis into the green zones as the leaves develop.

The evidence against the free movement of the common-mosaic virus and the yellowing strains in T.I. 448A tobacco, and the evidence for delayed movement of common-mosaic virus out of the initial infection sites in highly susceptible tobacco (4) and tomato (7), suggests that relatively few virus particles move long distances in the plant (13). It is to be expected that molecules or micellae of the immense size of the virus will not move about in the cytoplasm (15) and through the plasmodesmata and other channels as freely as sugars and other solutes.

It is of special interest that none of the mutants from the common-mosaic virus seemed to have greater invasive powers than the common-mosaic virus, and some of them seemed less invasive. None of these strains nor Nicotiana virus 1 induces local or systemic necrosis in T.I. 448A. Very small local necrotic lesions occurred on the leaves of Ambalema tobacco, but no systemic necrosis has been observed.

The strain of *Nicotiana glauca* used by the writer (8, 9) is variously similar to T.I. 448A and Ambalema tobaccos with respect to the resistance to the common-mosaic virus and yellow strains used in these studies. However, when a series of strains is tested on T.I. 448A and *N. glauca*, the exact order of virulence obtaining in one host is not reflected in the other. The results obtained with virus strains *BSY*, type *B* and "white" mosaic illustrate this point. Furthermore, the collection of *N. glauca* used does not react in the same way to different collections of the light-green-mosaic viruses that clearly belong in the common-mosaic group (8, 9). Such results make it rather clear that a single strain of virus cannot be used for indicating the precise mosaic-resistance-gene make-up in mosaic-resistant plants. Certain yellow-mosaic strains may serve as rough indicators of resistance to the wild or field-type of common mosaic virus, but the complete genetic picture cannot be obtained in this manner.

In the genus *Nicotiana*, the various gene complexes regulating symptom expressions induced by a given virus strain seem to show as much diversity as is shown among the various strains of the common-mosaic virus when compared on a given suitable genotype. In breeding work, these gene complexes seem to combine in so many different ways as to make it appear highly probable that all the strains of the common-mosaic virus would have to be used as testers in order to detect all of the gene complexes controlling the various types and degrees of disease expression in the genus.

Plants of T.I. 448A and Ambalema tobacco infected with Nicotiana virus 1 have never shown yellow-mosaic mutation spots (9) in the writer's tests, either in the field or in the greenhouse. Since all of the evidence available indicates that Nicotiana virus 1 interferes with the unlimited increase and spread of its mutants (9, 12), it seems highly probable that these resistant tobaccos may be regarded as very poor natural reservoirs for mutant viruses arising from Nicotiana virus 1. If at some future time it is discovered that Nicotiana 1 does give rise to mutants that suppress it, the problem will be changed and difficulties may be encountered with all of the types of resistant tobacco now in use.

Tobacco T.I. 448A appears to be one of the best genotypes, if not the best, now available for use in breeding for resistance against the common-mosaic virus and its mutants. Some of the genotypes isolated from the crosses studied by Nolla (16) and by Valleau (19) may be just as resistant as T.I. 448A, but this point can be settled only from the results of comparative tests, as the published results leave too many points in doubt.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

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A STUDY OF THE GENETICS OF SOROSPORIUM SYNTHERISMAE AND SPHACELOTHECA PANICI-MILIACEI[1]

W. J. MARTIN

(Accepted for publication October 1, 1942)

It is important for the plant breeder to know the range in variation in the fungi that cause plant diseases in order that he may work most efficiently in the production of improved varieties. A better knowledge of the extent and nature of variation in the smut fungi that attack grasses, therefore, should be beneficial to the plant breeder in the improvement of grasses.

There are over 100 species of smut fungi that attack approximately 250 species of grasses in the United States (1), but very little is known about variation in many of these species. Davis (5), Fischer (6, 7), and others (2, 11) have contributed greatly to our knowledge of some species that attack grasses. Martin and Kernkamp (15) found numerous biotypes in each of 5 species from grasses and reported the production of variants in monosporidial cultures of these smuts. Subsequently, *Sorosporium syntherismae* (Pk.) Farl. and *Sphacelotheca panici-miliacei* (Pers.) Bubak were studied more thoroughly to determine the extent and nature of variation resulting from mutation and hybridization. The results are given in this paper.

Sorosporium syntherismae is a head smut that has been reported on the following hosts: *Panicum agrostoides* Spreng., *P. capillare* L., *P. dichotomiflorum* Michx., *P. hirticaule* Presl., *P. stramineum* Hitchc. and Chase, *Cenchrus echinatus* L., *C. incertus* M. A. Curtis, *C. pauciflorus* Benth., and *C. tribuloides* L. *Sphacelotheca panici-miliacei*, also a head smut, occurs on *Panicum miliaceum* only (1, 24).

The literature on the genetics of the smut fungi has been summarized by Christensen and Rodenhiser (3) and by Miss Sampson (18); therefore, no general review of the literature is given, but pertinent literature is discussed in the text when deemed necessary.

MATERIALS AND METHODS

The collections of *Sorosporium syntherismae* were obtained from *Panicum capillare* and *Cenchrus pauciflorus*, two common hosts of that species; the collection of *Sphacelotheca panici-miliacei* came from *Panicum miliaceum*. The collections of the former conformed generally to Clinton's description of that species (4), while that of the latter agreed with Zundel's description (24).

[1] Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, June, 1942.

Paper No. 2030 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

Assistance in the preparation of these materials was furnished by the personnel of the Works Progress Administration, Official Project No. 165-71-1-124. Sponsored by the University of Minnesota, 1942.

The seeds of grasses used in the experiments were obtained from the following sources: *Panicum miliaceum* from A. C. Arny of the Division of Agronomy and Plant Genetics, University of Minnesota; *Panicum capillare* and *Cenchrus pauciflorus* from field collections made by the writer in 1940 at University Farm, St. Paul, Minnesota. All the seeds were treated in a

FIG. 1. Camera-lucida drawings of germinating chlamydospores of *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* stained with iron-alum haematoxylin.

1:250 solution of formaldehyde for 10 minutes, washed for 4 hours in running water, and then allowed to dry.

For inoculation purposes, monosporidial lines were grown in potato-dextrose broth containing 15 per cent dextrose; the high sugar content in-

duced a sporidial rather than a mycelial type of growth (13), which is highly desirable, especially when inoculations are made with a hypodermic syringe. Inoculations were made by injecting 10-day-old cultures into young seedlings, or else by soaking the seeds in the broth cultures for 24 hours shortly before planting.

EXPERIMENTAL RESULTS

Germination and Cytology of Chlamydospores

Chlamydospores of both *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* usually germinated by producing septate promycelia with lateral sporidia; however, in both species certain promycelial cells commonly produced hyphal branches instead of sporidia. Four-celled promycelia generally were uncommon in both species. The type of germination varied

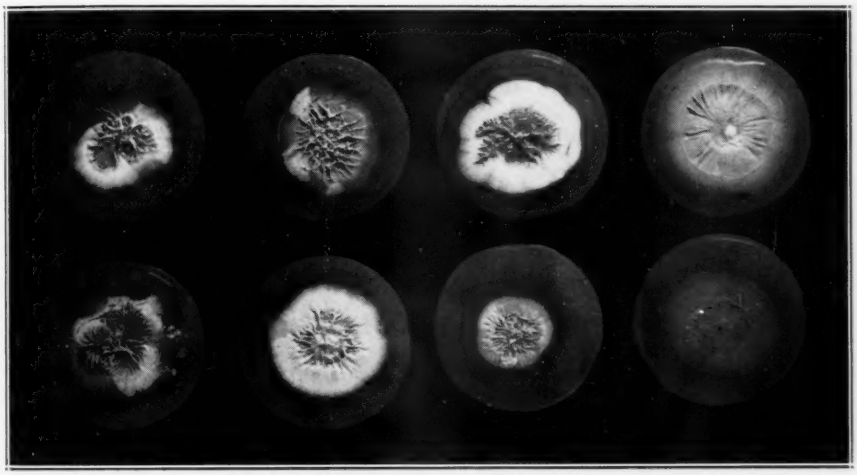

FIG. 2. Monosporidial cultures of *Sphacelotheca panici-miliacei* on potato-dextrose agar, showing a few cultural races. Note sectoring in the two cultures at left.

greatly among chlamydospores of the same collection, as described recently by Kernkamp and Petty (14) for *Ustilago zeae*. No distinguishing differences in the type of germination were observed between chlamydospores of *S. syntherismae* from *Panicum* and those from *Cenchrus*, although Norton (16) observed such differences in the collections with which he worked.

Harper's iron-alum haematoxylin technique was used (8) for studying the nuclear condition in the chlamydospores and sporidia. The chlamydospores, promycelial cells, and sporidia of both species were uninucleate (Fig. 1). Attempts to find meiotic figures were unsuccessful, but, since the writer observed that the very young chlamydospores are binucleate, and that infection occurs only when certain combinations of monosporidial lines are used in the inoculations, he assumed that the nuclear condition in these two smut fungi is similar to that in others, as reported by Wang (22) and others (see 3). That is, the sporidia normally are haploid and potentially gametic,

a dicaryotic condition is initiated at the time of infection, the diploid nucleus is formed at the time of maturation of the chlamydospore, and reduction division occurs when the chlamydospore germinates. The fusion of sporidia of opposite sex under cultural conditions, which is common in many of the smut fungi (see 3), was not observed with any degree of certainty, although repeated experiments over a wide range of conditions were made. However, sporidial fusions in *Ustilago zeae* have been seen but rarely, also (20).

Cultural Races, Mutation, and Intraspecific Hybridization

Approximately 150 monosporidial cultures of *Sorosporium syntherismae* from *Panicum capillare*, 130 of *S. syntherismae* from *Cenchrus pauciflorus*, and 120 of *Sphacelotheca panici-miliacei* from *Panicum miliaceum* were isolated. All of these isolates were grown on potato-dextrose agar and on two per cent malt agar in 250-cc. Erlenmeyer flasks. There were many cultural races in each species, and sectors frequently appeared in monosporidial isolates, as illustrated in figure 2. Such sector variants retained their distinctive characteristics when transferred to other flasks of agar, and could be maintained thus. Sectors often appeared in cultures that had originated from a mutant. Johnson, Rodenhiser, and Lefebvre (11) recorded a mutation in *S. syntherismae*, in which the mutant appeared in the form of buff sori on fall *Panicum*.

A comparison of the cultural characters of monosporidial isolates of *Sorosporium syntherismae* from *Cenchrus pauciflorus* with isolates of the same species from *Panicum capillare* showed that the isolates from certain collections from *C. pauciflorus* have a tendency to be somewhat different from those obtained from *P. capillare*, although this was not true for all collections.

Cultures obtained from single sporidia isolated from chlamydospores produced by crossing two monosporidial lines that originated from the promycelium of the same chlamydospore, differed strikingly in their cultural characters on potato-dextrose agar. Furthermore, none of the f_1 sporidial lines[2] was identical in cultural characters with either of the parental monosporidial lines, although some of the f_1 lines resembled one or the other parental line. That very few of the f_1 sporidial lines are identical with either of the parental lines also is true in the case of *Ustilago zeae* (20). Thus it appears that there are several factors governing cultural characters, and that there is a great deal of recombination of these factors, with segregation at the time of reduction division.

The Relative Pathogenicity of the Two Species

Cross inoculations were made with collections of *Sorosporium syntherismae* from *Cenchrus pauciflorus* and *Panicum capillare* and with *Sphacelotheca panici-miliacei* from *Panicum miliaceum*. Inoculations were made by soaking the seeds in a heavy chlamydospore suspension in water

[2] The term "f_1 sporidial line" is used in designating the monosporidial lines obtained from the F_1 chlamydospores; the small f is used since they are gametic lines.

for 24 hours, then the seeds were filtered off, allowed to dry, and planted. The results of these inoculations are summarized in table 1.

TABLE 1.—*Percentage of smut[a] produced on three species of grasses as a result of artificial inoculation with Sorosporium syntherismae and Sphacelotheca panici-miliacei*

Species of grass inoculated	Source of inoculum used and per cent smutted plants		
	S. syntherismae from *Cenchrus pauciflorus*	*S. syntherismae* from *Panicum capillare*	*S. panici-miliacei* from *Panicum miliaceum*
Cenchrus pauciflorus	1.8	1.2	0.0
Panicum capillare	6.3	8.7	0.0
Panicum miliaceum	16.8	12.6	65.7

[a] Percentage based on about 200 plants.

The percentage infection with *S. syntherismae* from either *Cenchrus* or *P. capillare* was very low in all the trials, particularly on *Cenchrus*. Attempts to obtain better infection by controlling various environmental factors, such as temperature and moisture, were unsuccessful. Johnson, Rodenhiser, and Lefebvre (11) reported good infection with *S. syntherismae* on *Panicum dichotomiflorum* Michx., but their methods were ineffective when tried with the two hosts used in this study.

Thus, *Sorosporium syntherismae* from either *Cenchrus pauciflorus* or *Panicum capillare* infected both of these species, and also *Panicum miliaceum;* while *Sphacelotheca panici-miliacei* infected only *P. miliaceum.* That *S. syntherismae* attacks *P. miliaceum* had not been clearly demonstrated previously.

Inoculations with monosporidial lines and with paired combinations of monosporidial lines of both *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* demonstrated that only certain paired combinations were capable of causing infection;[3] inoculations with other paired combinations and with individual monosporidial lines failed to cause infection. That paired combinations of opposite sex will cause infection, while paired com-

TABLE 2.—*Results of inoculating Panicum miliaceum with all possible combinations of eight monosporidial lines of Sorosporium syntherismae*

1	D_1	D_2	D_3	D_4	E_1	E_2	E_3	E_4
D_1	−	−	+	−	−	−	−	+
D_2	−	−	−	+	+	−	−	−
D_3	+	−	−	+	+	−	−	−
D_4	−	+	+	−	−	−	+	+
E_1	−	+	+	−	−	−	−	+
E_2	−	−	−	−	−	−	+	−
E_3	−	−	−	+	−	+	−	−
E_4	+	−	−	+	+	−	−	−

+ = caused infection.
− = did not cause infection.

[3] The criterion of infection as used here was the production of chlamydospores in the inflorescence or part of the inflorescence.

binations of the same sex will not, is in accordance with the behavior of other smut species that have been studied (3).

Results of inoculations with 8 monosporidial lines of *Sorosporium syntherismae* in all possible combinations showed the presence of 7 distinct "compatibility groups" in these 8 lines (Table 2). With the exception of lines $1D_3$ and $1E_4$ (alike in their ability to combine with other lines) each line differs in ability to combine with the other lines. Likewise, the results of inoculations with 4 monosporidial lines of *Sphacelotheca panici-miliacei* in all possible combinations demonstrated the presence of four compatibility groups in these four lines (Table 3). The occurrence of more than two compatibility groups in the smut fungi has been reported by several investigators (2, 3) and such groups have been referred to as sex groups.

TABLE 3.—*Results of inoculating Panicum miliaceum with all possible combinations of four monosporidial lines of Sphacelotheca panici-miliacei*

10C	1	2	3	4
1	−	−	+	−
2	−	−	−	+
3	+	−	−	+
4	−	+	+	−

+ = caused infection.
− = did not cause infection.

Interspecific Hybridization

Reports in the literature of the presence in nature of forms intermediate between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* suggested the possibility of the occurrence of hybridization between the two species. Zundel (24) states in a note below his description of *S. panici-miliacei* that certain collections on *Panicum miliaceum* closely resemble *S. syntherismae*. He states that these specimens have "larger, more irregular spores and, under an immersion-lens, often show more or less evident indications of verruculations in their walls." According to Winter (23), Fischer von Waldheim also observed such specimens and placed them in what is now called *S. panici-miliacei*. Winter states: "Die Sporen sind an allen von mir untersuchten Exemplaren glatt, während sie nach Fischer von Waldheim stachelig sein sollen." Certain of these specimens may have been *S. syntherismae* as the present writer showed that *P. miliaceum* can become infected by this species.

To test the possibility of interspecific hybridization between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei*, experiments were made in which a monosporidial line of one species was mixed with a monosporidial line of the other species, and the mixture injected into young grass seedlings by means of a hypodermic syringe. Seedlings of *Panicum miliaceum*, a host common to both species, and seedlings of *Panicum capillare*, a host of *S. syntherismae*, were inoculated with the interspecific combinations. As controls, seedlings were injected with each monosporidial line that entered

the combinations (Table 4). Certain of the interspecific combinations caused infection with the production of chlamydospores, while other combinations and the single monosporidial lines did not. This indicated that hybridization had occurred between the two species.

Inoculations with interspecific combinations then were made in which seeds of the White Ural variety of *Panicum miliaceum* were soaked in the various monosporidial combinations given in table 5. Each paired combination and each monosporidial line entering the combinations was used in inoculating lots of 175 seeds, each of which was then planted in two 9-foot rows in the field. The results of these inoculations are given in table 5.

The results of the inoculations in the field were similar to those in the greenhouse in that certain of the interspecific combinations caused infection,

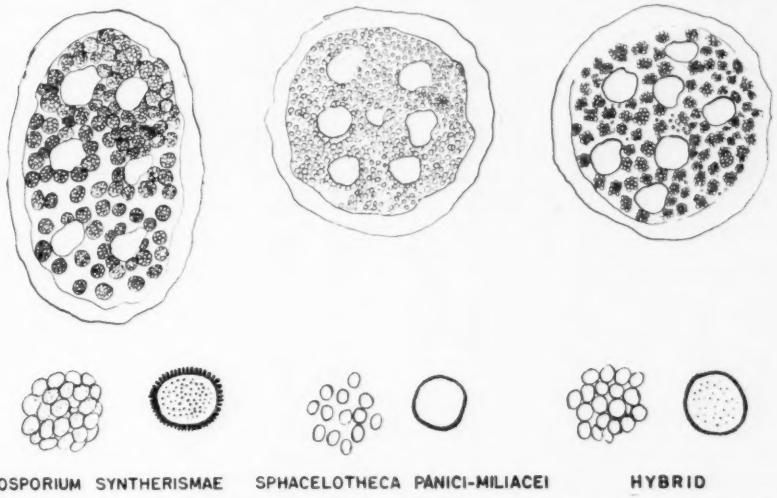

FIG. 3. Sori in cross section and spores of *Sorosporium syntherismae*, *Sphacelotheca panici-miliacei*, and the interspecific hybrid on *Panicum miliaceum*.

while other combinations and single monosporidial lines did not. Twenty-three of the 47 interspecific combinations used in inoculating plants of *Panicum miliaceum* were compatible and produced mature chlamydospores. Four of the 15 different interspecific combinations used in inoculating plants of *Panicum capillare* produced mature chlamydospores on that host. This indicated that interspecific hybridization had occurred, and to confirm this, a study was made of the F_1 chlamydospores and their progeny. The F_1 chlamydospores from certain of the crosses were used in inoculating *P. miliaceum* to obtain F_2 chlamydospores and certain of these in turn were used in inoculating plants to obtain F_3 chlamydospores. The inheritance of several characters was studied.

Inheritance of Characters in the Interspecific Hybrid

Wall Markings of Chlamydospores. Numerous intraspecific crosses (crosses between monosporidial lines of the same species) within both *Soro-*

sporium syntherismae and *Sphacelotheca panici-miliacei* were made, and in all cases those crosses within *S. syntherismae* resulted in the production of verruculose chlamydospores and those within *S. panici-miliacei* resulted in smooth chlamydospores. In all the interspecific crosses, the F_1 chlamydo-

TABLE 4.—*The results of inoculating plants of Panicum miliaceum and Panicum capillare with interspecific sporidial combinations of Sorosporium syntherismae × Spacelotheca panici-miliacei in the greenhouse*

Combinations *S. syn.* × *S. p–m*	*Panicum miliaceum*			*Panicum capillare*		
	Number of plants		Percentage smutted plants	Number of plants		Percentage smutted plants
	Inoculated	Smutted		Inoculated	Smutted	
$J_1 \times 5A_2$	32	0	0.0	35	0	0.0
$J_2 \times$ "	105	39	37.1	30	4	13.3
$J_3 \times$ "	42	0	0.0	36	0	0.0
$J_4 \times$ "	87	36	41.4	37	6	16.2
$J_5 \times$ "	45	12	26.7	30	3	10.0
$J_1 \times 5C_2$	26	0	0.0	18	0	0.0
$J_2 \times$ "	22	0	0.0	15	0	0.0
$J_3 \times$ "	28	0	0.0	14	0	0.0
$J_4 \times$ "	31	9	29.0	20	0	0.0
$J_5 \times$ "	29	8	27.6	20	0	0.0
$J_1 \times 5D_1$	21	0	0.0	16	0	0.0
$J_2 \times$ "	20	9	45.0	15	2	13.3
$J_3 \times$ "	25	0	0.0	19	0	0.0
$J_4 \times$ "	28	7	25.0	21	0	0.0
$J_5 \times$ "	26	8	30.8	22	0	0.0
$J_1 \times 10C_1$	29	10	34.5	.....	.....	.....
" $\times 10C_2$	18	0	0.0	.....	.....	.....
" $\times 10C_3$	22	3	13.6	.....	.....	.....
" $\times 10C_4$	26	1	3.9	.....	.....	.....
$J_2 \times 10C_1$	22	12	54.5	.....	.....	.....
" $\times 10C_2$	20	6	30.0	.....	.....	.....
" $\times 10C_3$	25	4	16.0	.....	.....	.....
" $\times 10C_4$	28	0	0.0	.....	.....	.....
J_1 Ck.	70	0	0.0	36	0	0.0
J_2 Ck.	77	0	0.0	30	0	0.0
J_3 Ck.	58	0	0.0	35	0	0.0
J_4 Ck.	79	0	0.0	33	0	0.0
J_5 Ck.	54	0	0.0	36	0	0.0
$5A_2$ Ck.	54	0	0.0	31	0	0.0
$5C_2$ Ck.	24	0	0.0	18	0	0.0
$5D_1$ Ck.	26	0	0.0	22	0	0.0
$10C_1$ Ck.	18	0	0.0	.....	.....	.....
$10C_2$ Ck.	16	0	0.0	.....	.....	.....
$10C_3$ Ck.	18	0	0.0	.....	.....	.....
$10C_4$ Ck.	22	0	0.0	.....	.....	.....

spores were verruculose and somewhat similar to those of the *S. syntherismae* parent (Fig. 3).

Eighty-nine F_2 sori from 3 interspecific crosses were examined microscopically, and the character of the spore walls recorded (Table 6). The ratio of sori containing verruculose spores to those containing glabrous spores approaches 3 : 1, and thus it appears that the factor for verruculose spores behaves as a simple dominant in this interspecific cross. This is in

accordance with other reports of interspecific hybrids between species of smut fungi having smooth and rough walls; *i.e.*, the factor for roughness is dominant over that for smoothness (3, 9, 17).

TABLE 5.—*The results of inoculating plants of Panicum miliaceum with interspecific sporidial combinations of Sorosporium syntherismae × Sphacelotheca panici-miliacei in the field*

Combinations *S. syn.* × *S. p–m*	Number of panicles		
	Total	Smutted	Percentage smutted
$J_1 \times 10C_1$	278	0	0.0
$J_2 \times$ "	261	0	0.0
$J_3 \times$ "	212	21	9.9
$J_4 \times$ "	221	0	0.0
$J_1 \times 10C_2$	203	0	0.0
$J_2 \times$ "	166	38	22.9
$J_3 \times$ "	205	0	0.0
$J_4 \times$ "	188	90	47.9
$J_1 \times 10C_3$	196	30	15.3
$J_2 \times$ "	180	0	0.0
$J_3 \times$ "	195	0	0.0
$J_4 \times$ "	160	0	0.0
$J_1 \times 10C_4$	151	13	8.6
$J_2 \times$ "	137	0	0.0
$J_3 \times$ "	186	22	11.8
$J_4 \times$ "	173	0	0.0
$J_1 \times 10D_1$	145	0	0.0
$J_2 \times$ "	192	163	84.9
$J_3 \times$ "	145	0	0.0
$J_4 \times$ "	183	66	36.1
$J_1 \times 10D_2$	228	0	0.0
$J_2 \times$ "	212	121	57.1
$J_3 \times$ "	241	0	0.0
$J_4 \times$ "	166	92	55.4
$J_1 \times 10D_3$	206	0	0.0
$J_2 \times$ "	226	178	78.8
$J_3 \times$ "	235	0	0.0
$J_4 \times$ "	214	133	62.1
$J_1 \times 10D_4$	198	0	0.0
$J_2 \times$ "	171	0	0.0
$J_3 \times$ "	192	0	0.0
$J_4 \times$ "	184	0	0.0
J_1 Ck.	210	0	0.0
J_2 Ck.	165	0	0.0
J_3 Ck.	197	0	0.0
J_4 Ck.	235	0	0.0
$10C_1$ Ck.	193	0	0.0
$10C_2$ Ck.	240	0	0.0
$10C_3$ Ck.	210	0	0.0
$10C_4$ Ck.	212	0	0.0
$10D_1$ Ck.	172	0	0.0
$10D_2$ Ck.	183	0	0.0
$10D_3$ Ck.	179	0	0.0
$10D_4$ Ck.	205	0	0.0

Inoculation of plants of *Panicum miliaceum* with smooth F_2 chlamydospores resulted in the production of sori that contained smooth spores; in-

TABLE 6.—*Segregation for spore wall markings in 89 F_2 sori from three interspecific crosses between Sorosporium syntherismae and Sphacelotheca panici-miliacei*

Interspecific cross *S. syn.* × *S. p–m*	Number of sori		
	Total	With smooth spore walls	With verruculose spore walls
$J_4 \times 10D_1$	23	5	18
$J_4 \times 10C_2$	28	6	22
$J_2 \times 10D_1$	38	9	29
Total	89	20	69

oculations with verruculose F_2 chlamydospores resulted in the production of some sori with verruculose spores and others with both verruculose and glabrous spores.

Attempts were made to mate the f_1 sporidial lines from the various interspecific courses with the parental monosporidial lines used in making the interspecific cross. Each of the 65 f_1 sporidial lines obtained was paired with each parental line, and plants of *Panicum miliaceum* were inoculated with combinations. Although repeated experiments were made, only 4 of these paired combinations caused infection. Two monosporidial lines from cross 22 (*Sorosporium syntherismae* $J_2 \times$ *Sphacelotheca panici-miliacei* $10D_1$) were compatible with the *S. panici-miliacei* parent, and two other lines were compatible with the *S. syntherismae* parent. The two crosses involving an f_1 sporidial line and a line from the Sorosporium parent resulted in the production of verruculose spores, as was expected; the two crosses with the *Sphacelotheca* parent yielded smooth spores. Numerous attempts were made to obtain a larger number of such crosses to determine whether the expected 1:1 ratio of verruculose to glabrous spores would result from crossing f_1 sporidial lines with the parental line from the *Sphacelotheca* parent. For some unknown reason, however, only 4 of the 130 paired combinations caused infection.

Size of Chlamydospores. The F_1 hybrid chlamydospores from interspecific crosses were intermediate in size as compared with chlamydospores from intraspecific crosses in *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei,* respectively, as is shown in table 7. The difference in spore size for the two species, however, is small, and attempts to follow this character in the F_2 generation were unsuccessful. Nevertheless, it was possible to find the larger, smooth spores and also the smaller verruculose spores in the F_2

TABLE 7.—*The size of chlamydospores*[a] *of Sorosporium syntherismae, Sphacelotheca panici-miliacei, and a hybrid between the two species*

Smut species or hybrid	Range in microns	Average in microns
Sorosporium syntherismae	8.4–12.0 × 7.0–10.9	9.8 × 8.5
Sphacelotheca panici-miliacei	7.0–10.2 × 6.0–10.1	8.2 × 7.8
Hybrid (*S. syn* $J_4 \times$ *S. p–m.* $5A_2$)	7.0–11.5 × 6.4–10.5	8.7 × 7.9

[a] Two hundred chlamydospores of each species and of the hybrid were measured.

generation of the interspecific crosses, while in the intraspecific crosses the smaller spores (those of *S. panici-miliacei*) are smooth and the larger spores (those of *S. syntherismae*) are verruculose. Thus it appears that the factors for wall markings and size of the chlamydospore are inherited independently in the interspecific hybrid, or, if they are linked, crossing-over occurs frequently.

Germination of Chlamydospores and Viability of Sporidia. The F_1 hybrid chlamydospores germinated by producing septate promycelia with lateral sporidia, as do chlamydospores of both parent species. There was, however, a difference among chlamydospores of both the smut species and the hybrid in the time required for germination. Two different interspecific hybrids were compared with the two parent species: the chlamydospores from the two interspecific hybrids required 10 and 11 hours for germination, whereas those of *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* required 20 hours and 7 hours, respectively.

Most of the interspecific hybrid chlamydospores germinated and produced primary sporidia, a high percentage of which, however, failed to bud. In both parent species over 90 per cent of the single sporidial isolates developed into microscopic colonies, whereas less than 10 per cent of over 700 single sporidial isolates from the F_1 hybrid chlamydospores developed into colonies. Many of the sporidia that were isolated gradually underwent lysis without budding; others budded a few times, then underwent lysis, while a few others budded and developed into colonies. Not a single complete set of monosporidial cultures from a hybrid chlamydospore was obtained, even though over 100 such complete sets were isolated. This low viability of f_1 sporidia from interspecific crosses in the smut fungi has been reported previously (9, 10), and it appears to be a type of hybrid sterility. Sporidia from the F_2 hybrid chlamydospores did not undergo lysis.

Pathogenicity. The interspecific dicaryophytes, *i.e.*, those dicaryophytes that arose from the union of a haploid line of one species with a haploid line of the other, attacked both *Panicum miliaceum* (a host of both species of smut) and *Panicum capillare* (a host of *Sorosporium syntherismae* only). Thus the interspecific dicaryophyte had the combined pathogenic capabilities of both species of smut.

The interspecific dicaryophytes originating from different monosporidial combinations differed strikingly in their ability to produce smut on the White Ural variety of *Panicum miliaceum* when inoculated in the field (See Table 5 and Fig. 4). The percentage of smutted heads produced by the different dicaryophytes ranged from 8.6 per cent ($J_1 \times 10C_4$) to 84.9 per cent ($J_2 \times 10D_1$). (See Fig. 4.)

Experiments in the greenhouse in which 6 varieties of *Panicum miliaceum* were inoculated with 6 interspecific combinations confirmed these differences in the pathogenicity of different interspecific dicaryophytes. The results of a series of inoculations in the greenhouse are given in table 8. The dicaryophytes arising from combinations $J_2 \times 10D_1$, $J_2 \times 10D_2$, $J_2 \times 10D_3$, and

$J_2 \times 10C_2$ produced the highest percentage of smut on all the varieties; the other two dicaryophytes produced a relatively low percentage or no smut, depending on the variety. The varieties Early Fortune and Brown Ottawa were resistant to all the dicaryophytes, the highest percentage of smut produced on these 2 varieties being between 10 and 15 per cent by the dicaryophytes that were the most pathogenic on the other varieties.

It is not surprising to find such great differences in the pathogenicity of interspecific dicaryophytes, however, because such differences have been reported in intraspecific dicaryophytes of certain other smuts (3, 19, 20).

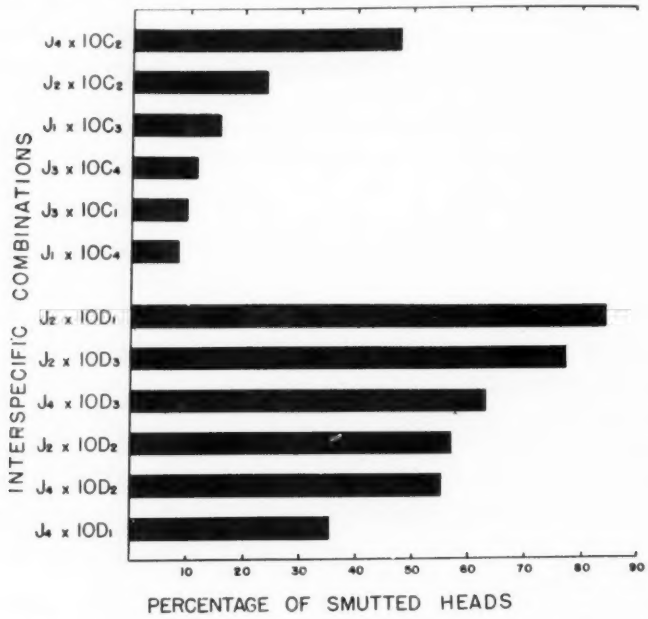

FIG. 4. Percentage smut produced on the White Ural variety of *Panicum miliaceum* by twelve interspecific dicaryophytes of *Sorosporium syntherismae* × *Sphacelotheca panici-miliacei* in the field.

The differences in the pathogenicity of dicaryophytes are important in classifying varieties as to resistance or susceptibility to smut. For example, varieties of *Panicum miliaceum* would be classed as resistant to interspecific dicaryophytes if their reaction to the 2 combinations $J_1 \times 10C_3$ and $J_3 \times 10C_1$ only was considered; but these same varieties would be classed as very susceptible on the basis of their reaction to the combination $J_2 \times 10C_2$ (Table 8).

Other Characters. The F_1 interspecific hybrid was intermediate between the two parent species in spore arrangement and also in type of sori (Fig. 3). The spores of the F_1 hybrid were somewhat loosely grouped but not in definite spore-balls, those of the Sorosporium parent were arranged in definite spore-balls, while those of the Sphacelotheca parent were single. The sori of the F_1 hybrid were ovoid to elongate and certain ones had rather definite central columellae, while others did not; the sori of the Sorosporium

parent were elongate and had no central columella; the sori of the Sphacelotheca parent were ovoid to spherical and had definite central columellae.

The cultural characters of the f_1 sporidial lines from interspecific cross 20 (*Sorosporium syntherismae* $J_4 \times$ *Sphacelotheca panici-miliacei* $5A_2$) were

TABLE 8.—*Percentage of smut produced by six different interspecific sporidial combinations of Sorosporium syntherismae × Sphacelotheca panici-miliacei on six varieties of Panicum miliaceum in the greenhouse*

Variety	Interspecific combination *S. syn.* × *S. p–m.*	No. plants emerged	No. plants smutted	Percentage smutted plants
White Ural	$J_2 \times 10D_1$	46	32	69.6
	$J_2 \times 10D_2$	52	24	46.2
	$J_2 \times 10D_3$	40	23	57.9
	$J_3 \times 10C_1$	45	1	2.2
	$J_2 \times 10C_2$	42	35	83.3
	$J_1 \times 10C_3$	34	6	12.2
	Check	54	0	0.0
Early Fortune	$J_2 \times 10D_1$	52	6	11.5
	$J_2 \times 10D_2$	41	3	7.3
	$J_2 \times 10D_3$	64	6	9.3
	$J_3 \times 10C_1$	49	0	0.0
	$J_2 \times 10C_2$	46	6	13.0
	$J_1 \times 10C_3$	41	1	2.4
	Check	44	0	0.0
Black Voronezh	$J_2 \times 10D_1$	56	44	78.6
	$J_2 \times 10D_2$	55	39	70.9
	$J_2 \times 10D_3$	83	54	65.0
	$J_3 \times 10C_1$	81	12	14.8
	$J_2 \times 10C_2$	66	48	72.7
	$J_1 \times 10C_3$	62	12	19.3
	Check	50	0	0.0
Brown Ottawa	$J_2 \times 10D_1$	60	8	13.3
	$J_2 \times 10D_2$	47	6	13.6
	$J_2 \times 10D_3$	53	5	9.4
	$J_3 \times 10C_1$	73	0	0.0
	$J_2 \times 10C_2$	49	7	14.3
	$J_1 \times 10C_3$	57	0	0.0
	Check	63	0	0.0
Red Turghai	$J_2 \times 10D_1$	48	18	37.5
	$J_2 \times 10D_2$	55	19	34.5
	$J_2 \times 10D_3$	48	20	41.6
	$J_3 \times 10C_1$	43	0	0.0
	$J_2 \times 10C_2$	51	33	64.7
	$J_1 \times 10C_3$	54	3	5.5
	Check	45	0	0.0
Early Manitoba	$J_2 \times 10D_1$	44	44	100.0
	$J_2 \times 10D_2$	32	27	84.4
	$J_2 \times 10D_3$	37	29	78.8
	$J_3 \times 10C_1$	40	6	15.0
	$J_2 \times 10C_2$	31	29	93.5
	$J_1 \times 10C_3$	34	15	44.1
	Check	24	0	0.0

compared with those of the parental lines. Certain characteristics of both parental lines were found in the f_1 lines, but no exact duplicate of either of the parents was found in the 18 monosporidial lines studied. This was not surprising, however, since parental types were not recovered from the intra-

specific crosses reported at the beginning of this paper. The various biotypes present in the lines studied are illustrated in figure 5. The lines

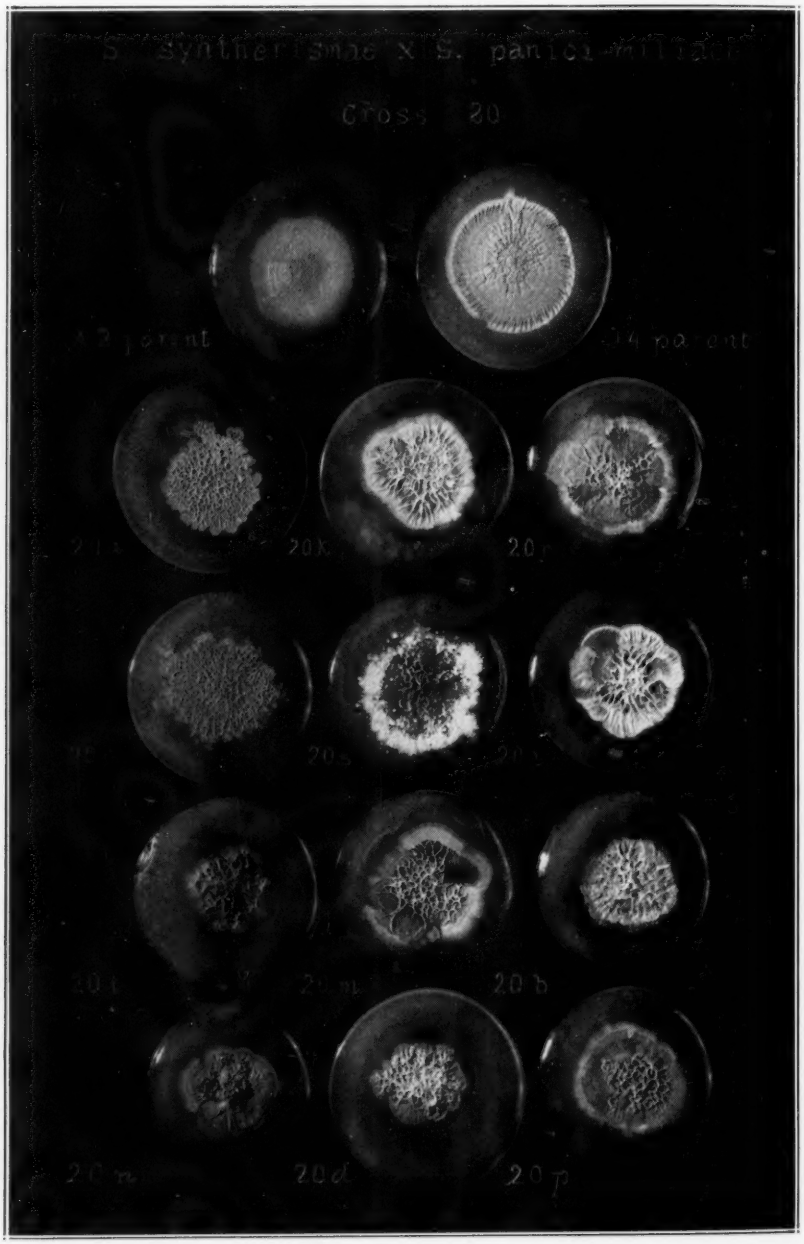

FIG. 5. Cultural races in the f_1 sporidial lines from an interspecific cross between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei*.

b, i, r, and p resemble more closely the Sorosporium parent (J_4), and the lines a, c, and t resemble more closely the Sphacelotheca parent ($5A_2$).

Evidence of Hybridization Occurring in Nature

Several experiments were made in which seeds of *Panicum miliaceum* were inoculated with a mixture of chlamydospores of both species of smut and with chlamydospores of each species individually. In one series of these experiments about 50 per cent smut (based on about 100 plants) was produced by each of the species and by the mixture of both species. Hybrid chlamydospores were found in two of the galls produced on plants that had been inoculated with the mixture. In both cases these hybrid spores were found in galls that contained over 50 per cent smooth spores (those of *Sphacelotheca panici-miliacei*), and there was no question that they were hybrid spores. This is further evidence that hybridization between the two species of smut could easily occur in nature where the two smuts occur in the same locality. Kammerling (12) reported that he found in nature a hybrid between *Ustilago longissima* and *U. longissima* var. *macrospora*. He, also, was able to produce experimental hybrids between these two smuts.

SUMMARY AND CONCLUSIONS

The life cycle and nuclear condition in *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* are similar to those of most smut fungi that have been studied; the sporidia are haploid and potentially gametic, infection occurs in the seedling stage of the host, the parasitic phase is dicaryotic, mature chlamydospores are diploid, and reduction-division occurs during germination of chlamydospores.

Much the same type of variation occurs in both *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* as occurs in other smut fungi that attack graminaceous crop plants. There are numerous biotypes within each species, and new biotypes result from mutation and hybridization.

The collections of *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* studied by the writer are definitely distinct species. They differ in type of sorus as well as in size, arrangement, and markings of the chlamydospores. The writer has not collected intergrades between the two species, as reported by Zundel and others (24, 23), possibly because collections were not made over a sufficiently wide area. The results from cross inoculations with the two species demonstrated that *S. panici-miliacei* attacked only *Panicum miliaceum*, which is in agreement with reports in the literature (1, 24). But it was demonstrated that *S. syntherismae* attacks *P. miliaceum* as well as *Panicum capillare*. Neither Zundel (24) nor Barnhart (1) lists *P. miliaceum* as a host of *S. syntherismae*, although Zundel does state that certain collections on *P. miliaceum* closely resembled *S. syntherismae*. From the writer's results it appears that the abnormal specimens of Zundel (24) and others (23) might have been specimens of *S. syntherismae* or hybrids between this species and *S. panici-miliacei*. Chlamydospores of hybrids between the two species certainly fit the description of the abnormal specimens of these two investigators. Furthermore, hybridization readily occurred between the two species under experimental conditions, some of

which do not appear to be far different from conditions that might occur in nature.

The interspecific hybrid between *Sorosporium syntherismae* and *Sphacelotheca panici-miliacei* was more or less intermediate between the two parent species in many characters, including type of sorus, size of spores, markings of the spore walls, arrangement of the spores, time required for germination of the spores, and pathogenicity. Size of spores and markings of the spore walls are controlled by different factors, which appear to be inherited independently. The factor for verruculose spore walls behaves as a simple dominant over that for smooth spore walls. Fewer than 10 per cent of the f_1 hybrid sporidia were viable, which has been reported also for other interspecific crosses in the smut fungi (9, 10).

The interspecific dicaryophytes produced in the study were capable of attacking both *Panicum miliaceum* and *Panicum capillare*. Furthermore, there were marked differences in the pathogenicity of dicaryophytes arising from different interspecific combinations. Other investigators (3, 9, 10, 12, 19, 20, 21) have emphasized the importance of interspecific, as well as intraspecific, hybridization in giving rise to new biotypes in the smut fungi.

UNIVERSITY FARM,
ST. PAUL, MINNESOTA.

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SEVERITY OF CURLY TOP IN TOBACCO AFFECTED BY SITE OF INOCULATION

W. C. Price

(Accepted for publication November 6, 1942)

Evidence for passive immunization of tobacco (*Nicotiana tabacum* L.) from the virus of sugar-beet curly top (*Chlorogenus eutetticola* H.) was reported by Wallace (20) in 1940. It was observed that grafting with cions from tobacco plants that had recovered from curly top transmitted only a mild disease, whereas the insect vector (*Eutettix tenellus* (Baker)) transmitted a severe disease from the same source plants. Wallace concluded that the grafting process transmitted protective substances along with the virus, whereas the insect vector transmitted only the virus. The studies herewith presented dealt with the effect of route of infection on severity of curly-top symptoms in tobacco. In these it was found that the grafting process does not always transmit a mild disease from recovered plants and that the insect vector may transmit either a mild or a severe disease, depending upon the portion of the test plant on which it is allowed to feed. The results, therefore, do not support the antibody hypothesis, but rather indicate that the explanation for production of only mild symptoms by grafting must be sought elsewhere.

REVIEW OF LITERATURE

Of the many species of plants susceptible to curly-top virus, tomato (*Lycopersicon esculentum* Mill. and *L. pimpinellifolium* Mill.) and tobacco (*Nicotiana tabacum*) have been shown to recover from the acute form of disease produced by this virus. When curly-top virus is taken to tomato, it induces clearing of veins of the newly formed leaflets and severe curling and distortion of the youngest leaves; affected plants gradually wilt, turn yellow, and die within a few weeks after infection (4). It was observed by Lesley (7), however, that tomato plants in an advanced stage of curly top sometimes recovered, especially in late summer and fall. This recovery was probably due to the escape of portions of the tomato plants from the curly-top virus, since some of the new shoots from recovered plants later became severely diseased and since, in one instance, exposure of a recovered plant to viruliferous insects resulted in development of severe curly top in this plant. A different type of recovery was observed by Lesley and Wallace (8) in plants of certain races of tomato. In some of these plants, relatively healthy shoots arose from the leaf axils and the plants then grew almost normally until maturity, although they showed mild symptoms of disease and continued to harbor the curly-top virus. In most instances, reinoculation of the recovered plants failed to induce further symptoms. In a few cases, however, recovered plants relapsed and suffered a second attack of curly top.

Sugar-beet curly-top virus was first transmitted to tobacco by Severin (13) in 1929. In 1936 Bennett and Esau (3) reported that tobacco plants infected with the virus showed a marked recovery from the initial disease, the new growth appearing healthy or developing only mild symptoms. The recovery was apparently due to a reaction on the part of the plant itself and not to an attenuation of virus. Wallace (19) confirmed the observations of Bennett and Esau and stated that the new, healthy-appearing growth may arise either from the axillary or terminal buds. He showed further that recovered tobacco plants may be grown from cuttings without again showing severe symptoms, even though reinoculated with the virus. This acquired immunity from curly top is thus similar to that observed in tobacco ring spot and certain other plant virus diseases (10).

Wallace (20) not only found, as previously mentioned, a difference between symptoms following insect transmission and those resulting from grafting with cions from recovered plants, but he also observed that the severity of disease induced by grafting was dependent upon the stage of infection of plants from which cions were taken. Severe symptoms developed in healthy tobacco plants grafted with scions taken from plants 5 days after they had been inoculated with curly-top virus, mild symptoms were obtained on plants grafted with cions taken from plants exposed 20 days previously to viruliferous insects, and symptoms of intermediate severity developed when cions from plants inoculated 10 and 15 days previously were used. These results led to the conclusion that "the reaction leading to recovery took place at an early stage following infection and that it was completed or well advanced before the plants began to recover." The results were thus taken as corroborative evidence that protective substances were transmitted from recovered plants by means of grafting.

Still further evidence for the antibody hypothesis was reported by Wallace (21) in 1942. According to him, the clonal progeny of tomato plants that were grafted with cions from recovered tobacco plants showed a high degree of protection from curly top when grown under field conditions. Such grafted plants, when reinoculated with curly-top virus, were either unaffected, mildly affected, or severely affected, depending upon the strain of virus used for reinoculation. Thus, while grafting with cions from recovered tobacco plants gave protection against the strain of virus originally used to inoculate the tobacco plants from which cions were taken, it did not give protection against other closely related strains of the virus.

MATERIALS AND METHODS

In the first attempts to transmit sugar-beet curly-top virus to Turkish tobacco, the virus either was not transmitted or failed to induce obvious signs of disease. The strain of virus available at that time was, therefore, not suitable for the purpose. Later, a strain that did induce severe symptoms in Turkish tobacco was kindly supplied by Dr. Wallace. This strain apparently is identical with the one with which he worked and has been used in all the experiments to be reported.

Turkish tobacco and tomato plants were, for the most part, grown in 6-inch porous clay pots in a greenhouse held above 70° F. They grew more rapidly in the late spring, summer, and early fall than during the winter months, but the symptoms induced on them did not appear to be correlated with the season. The greenhouse was fumigated at frequent intervals to control insects.

The leaf hoppers used for transmission of the sugar-beet curly-top virus were maintained on healthy or curly-top-diseased sugar beets in a separate greenhouse or in a laboratory window until placed on tobacco or tomato test plants. They were handled by means of a suction tube similar to that devised by Kunkel (6) and caged on the test plants or portions of them by means of celluloid cages similar to those described by Shapovalov (14). *Eutettix tenellus* will feed and survive on tobacco only for about a day. It was, therefore, the practice in this work to place from 6 to 15 leaf hoppers on the test tobacco and tomato plants for one day and then remove them to sugar beets for at least a day before again using them for tobacco or tomato. In this way an infective colony could be used for a number of successive transmissions.

Two types of grafts were employed. One was the cleft graft and the other will be referred to as a lateral graft. In the former type the cion consisted of a portion of the stem from 3 to 5 inches in length, with most of the leaves removed, and cut to a wedge shape on the basal end. For the stock vigorous plants were decapitated 6 or more inches above the soil level and the stub split with a sharp knife for a distance of about 2 inches. The cion was inserted into the split stem and the two bound together. A paper bag was then placed over the top of the plant and left on for 1 or 2 days. In the lateral graft the cion was a 1-inch slice of stem, containing a bud, from which the pith had been removed or, in some cases, a section of stem, with leaves, from the top of a plant, sliced diagonally at the base. The cion was bound to a section of stem of the stock from which the epidermis had been removed or scarified. The binding material, "Sterilastic," was removed or cut away after 2 or 3 weeks or was left on and allowed to disintegrate.

DESCRIPTION OF SYMPTOMS

The terms severe, mild, moderate, and moderately severe will be used to describe the symptoms obtained in tobacco in the experiments to be reported. These are relative terms used merely to indicate the degree of severity of symptoms. They refer to syndromes, which may be described as follows:

Severe: The first sign of disease is clearing of veins in young, partly expanded, apical leaves. All the main veins become bright yellow and thus make the venation conspicuous. Leaves showing this symptom usually curl upward at the basal edges. Leaves produced later turn downward from the edges. Usually 8 to 10 or more leaves show this downward curling, most of them without marked clearing of veins. The veins expand less than the

interveinal tissue, giving the leaf a decidedly puckered appearance. Many of the leaves are about 1/20 their normal size and rolled into compact masses or balls. They frequently retain their normal green color; sometimes they are even darker green than normal. Older leaves, those first affected, or those that were more fully expanded at the time they were invaded, may yellow and die. Internodes are markedly shortened; a section of stem 1 inch long may bear 5 or 6 leaves as compared with only 1 or less for the normal plant. Thus, the apex of the plant becomes rosetted. The stem may grow more on one side than on the opposite, curling downward or becoming twisted and distorted. As the plant matures, the rosetted appearance may become less marked due to some unfolding of affected leaves and lengthening of the shortened internodes, but the affected portions never appear normal or even nearly so. Axillary buds sometimes are stimulated to grow, but shoots produced from them remain small, become markedly curled and distorted, and often die. When the plant is infected late, the flower stalk may show similar symptoms. The flowers are bunched, the sepals crinkled or puckered, and the corolla misshaped. After a variable period of from 1 to 3 months, an axillary bud or the terminal bud may send out a healthy-appearing shoot. This shoot continues to grow and never shows the severe symptoms characteristic of the onset stage, although it may at times produce leaves with mild clearing of veins, curling, or puckering. The plant has thus recovered from the acute form of the disease.

Mild: The symptoms first appear as clearing of veins, as in the severe type, but the clearing usually is not so pronounced. The leaf edges curl upward at the base. Leaves so affected may later curl downward and show crinkling of the tissues between the lateral veins. They retain their normal color or may become slightly darker green than normal. Usually only 2 or 3 leaves show such manifestations; they are reduced only slightly in size, perhaps to ¾ normal, and become more nearly normal as they mature. The internodes are not appreciably shortened and the plant does not assume a rosetted appearance. After the initial shock the new growth appears almost normal. It may often be differentiated from healthy growth by its slightly darker color and by the appearance of crinkled or puckered areas between lateral veins.

Moderate and Moderately Severe: There are various gradations between the two extremes, mild and severe, of symptom expression. The number of leaves affected may vary from 2 to 15 or more. Affected leaves may be curled more than in the mild type but less than in the severe. Also, the degree of shortening of the internodes may vary. The terms moderate and moderately severe are used to indicate degrees of severity of symptoms between the mild and severe types. Whether the disease picture be classified as severe or moderately severe, as mild or moderate, is a matter of judgment. An affected plant may be classified as moderate on one occasion and mild or moderately severe, or even severe, on the next examination; that is to say, the plants may have become more or less severely diseased in the

interval between examinations. For this reason, affected plants should be examined at frequent intervals over a period of 2 or more weeks after they first show symptoms before being classified according to symptom type.

EXPERIMENTAL

Tests with Turkish Tobacco

Symptoms Produced by Cleft-grafting with Cions from Newly Infected and from Recovered Plants. Turkish tobacco plants, grown in 6-inch pots to a height of 18 to 24 inches in one test and 12 to 16 inches in another, were cut back to a height of about 8 inches and cleft-grafted either with cions from plants that had recovered from curly top or with cions from recently diseased plants (exposed 10 days previously to viruliferous insects). In the first test symptoms appeared on some plants 8 days after grafting but failed to appear on more than half of the plants within one month after

TABLE 1.—*Symptoms produced in Turkish tobacco plants after cleft-grafting with cions from recently diseased and recovered plants*

		Plant No.											
		1	2	3	4	5	6	7	8	9	10	11	12
Test 1													
R[a]	B	+[b]	+++	−	−	−	−	+++	−	−	+++	+++	−
	A	+	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
D	B	++++	−	−	+++	−	++++	+++	−	+	−	−	−
	A	+	++++	++++	++++	++++	+	+++	+	++++	++++	++++	++++
Test 2													
R		+++	++	+	+	−	++	+++	+	−			
D		++++	++++	+	+++	+++	−	++++	−	−			

[a] R, cions from recovered plants; D, cions from recently diseased plants; B, before being pruned back; A, after being pruned back.

[b] ++++, severe symptoms; +++, moderately severe symptoms; ++, moderate symptoms; +, mild symptoms; −, no symptoms.

grafting. The plants were therefore pruned back to the first bud or shoot above ground level and held under observation for another month. Plants in the second test were grown for a period of 6 weeks after grafting and observed for signs of disease at frequent intervals during this period. The symptoms produced in the two tests are summarized in table 1. The results were unexpected, on the basis of what had previously been reported (20). There was no clear-cut difference between symptoms induced by the two types of cions, although there seemed to be a tendency for cions from recently diseased plants to induce somewhat more severe symptoms on the average than those from recovered plants. The failure of many plants to show symptoms at all was obviously due to the failure of virus to reach the growing tips where its effects could become apparent. If antibodies or protective substances were transferred from cion to stock, it would seem that the stock should be protected and show only mild symptoms. Since this was not the case, the results could not be construed as bringing evidence for the presence of such substances.

Plants that were cleft-grafted with scions from recovered or recently diseased plants usually developed more than one axillary shoot. In many instances different shoots on the same grafted plant showed different symptoms. While some shoots developed severe symptoms, others on the same plant developed none at all or only mild symptoms.

On many occasions, tobacco plants that had fully recovered from a severe attack of curly top were pruned back and the axillary buds forced into growth. This growth in some cases appeared normal, but in most instances it showed mild symptoms of curly top for a short time and then appeared practically normal. In no instance did severe curly-top symptoms develop when fully recovered tobacco plants were pruned back.

The behavior of one plant, number 7 of test 2, grafted with a cion from a recently diseased plant, is worthy of further comment. The cion made

TABLE 2.—*Symptoms produced in Turkish tobacco plants after grafting laterally with cions from recovered and recently infected plants*[a]

Test No.		Plant No.											
		1	2	3	4	5	6	7	8	9	10	11	12
1	R	++++	++	++	++	−	−	+++	++	+++	++	+++	+++
	D	++++	+++	+++	++++	++++	+++	++++	++	−	++++	++++	+++
2	R	+	−	+	+	−	++++	+	−	−	+	+	−
	D	++++	++	+	++++	++	+	+	−	+	−	−	+
3	D_1	+++	−	−	+	−	++	++++	−	+	++++	++	+
	D_2	−	++++	−	−	−		+	+++	−	−	−	−
	D_3	−	−	++++	+++	++++	−	−	−	+++	−	−	−

[a] For explanation of symbols, see footnotes to table 1. D_1, D_2, and D_3, cions from plants 20, 15, and 10 days, respectively, after exposure to viruliferous insects.

a quick union with the stock and grew rapidly. Its growth apparently inhibited the production of axillary shoots, since these developed very slowly. The symptoms produced on the cion were at first severe, then became mild, and finally disappeared as the cion recovered. Six weeks after grafting the cion had grown to a height of 11 inches, most of it being composed of healthy-appearing foliage. Two shoots had grown from axillary buds at the base and both of these showed severe symptoms. The potential supply of antibodies in this recovered cion was indeed large as compared with the supply in a newly grafted cion. If such substances were present and capable of moving from one part of a plant to another, it is surprising that they did not inhibit the symptoms on the axillary shoots produced after the cion had recovered. It is to be inferred that such substances either were not present or, if present, were incapable of moving in this instance.

Symptoms Produced by Lateral Grafting with Cions from Newly Infected and from Recovered Plants. The possibility existed that the rapid growth of axillary shoots following cleft-grafting in some way may have overcome the influence of any protective substances that had been transmitted through the graft union. The lateral graft, which does not stimulate

axillary buds to grow, was used in a series of tests with the results summarized in table 2. Here again the data indicated a slight difference between symptoms produced by grafting with cions from recovered plants and from recently infected plants. The difference, however, was not consistent and can possibly be attributed largely to chance. Moreover, it is apparent that many plants grafted with cions from recovered plants came down with severe or moderately severe symptoms, while a smaller number showed mild symptoms. It could not be concluded that the experiment demonstrated the movement of antibodies from cion to stock.

Attempt to Isolate Mild and Severe Strains by Selection. In contrast to the variable symptoms induced by grafting, plants inoculated by allowing viruliferous insects to feed on them invariably came down with severe curly top. Was this difference due to the transmission of antibodies by the grafting process or was it due, for example, to the presence of a mixture of virus strains in the inoculum? It could be reasoned that, following grafting, one or another strain might predominate fortuitously and that, on the other hand, the vector tended to select the more severe strain without entirely eliminating the others. To test this possibility, an experiment was designed to see whether or not severe and mild strains could be obtained by a process of selection and elimination over a period of time.

Two plants from a previous experiment were selected as a source of cions; one had developed mild symptoms after grafting and the other had developed severe symptoms. Six cions were taken from each plant and grafted separately to Turkish tobacco plants. The cions from the severely affected plant produced severe symptoms in one stock and mild or moderate ones in the others; the plant with severe symptoms was selected for further grafting. The cions from the mildly affected plant produced mild symptoms in one plant, moderate symptoms in 3, and none in 2; the mildly affected stock was selected for additional grafting. This process was continued until each selection had been passed through 5 successive transfers by grafting. At the end of this period there was no evidence that there had been a selection of strains from a complex, and the 2 isolates showed about the same variation in symptoms as they had in the beginning. This experiment, therefore, brought no evidence that the difference between symptoms produced following grafting and those following insect transmission could be accounted for on the basis of a mixture of virus strains.

Influence of Source Plants and Position from Which Cions Were Taken. While the data so far presented indicated a significant difference between transmission by grafting and by means of the insect, this difference was by no means so clear-cut as that obtained by Wallace (20). Moreover, the results with grafting were erratic; grafting with cions from recently diseased or recovered plants might result either in a mild or a severe disease, and it was impossible to predict ahead of time which result would be obtained. If proof of the antibody hypothesis were to be secured, it was necessary to obtain consistent results. What factors could account for the

variation? Was it possible that different recovered plants or parts of these plants differed in their antibody content or in their ability to transmit antibodies to a plant on which they were grafted? An experiment designed to give an answer to the latter question was carried out on two different occasions. Recovered plants were selected and 5 cions were taken from each, 1 from the top of the plant, 1 from near the base, and 3 from intermediate positions. Each cion was cleft-grafted to a large healthy plant in a 6-inch pot. Table 3 summarizes the results obtained. The 5 cions from one recovered plant all transmitted a mild disease; the 5 cions from 2 other recovered plants transmitted a severe disease in each instance; the 5 cions from the other 13 plants transmitted severe, mild, or intermediate symptoms indiscriminately. There appeared to be no correlation between cion

TABLE 3.—*Symptoms produced in Turkish tobacco plants after cleft-grafting with cions from different portions of recovered plants*[a]

Cion position		Plant No.									
		1	2	3	4	5	6	7	8	9	10
Top	A	++	+++	++++	++	+	++++				
	B	+	++	+	+	+	++++				
	C	−	+	+++	+++	+++	+				
	D	++++	+++	+	+++	++	++				
Base	E	++	+++	+++	++	++++	−				
Top	A	+	+	++++	++++	+	++	++++	++++	+++	+
	B	+	+	++	+	+	+	++++	++++	++++	++++
	C	+	+	++++	++	++	+	++++	++++	+	++++
	D	++++	+	++++	++	++	+	++++	++++	++++	++++
Base	E	++++	+	++++	++++	+	++++	++++	++++	+	++++

[a] For explanation of symbols, see footnotes to table 1.

position and type of disease transmitted. It was concluded that some as yet unknown factor was responsible for the variation observed.

Rate of Appearance of Symptoms after Grafting. It has been shown by Bennett (1) and confirmed in the present work that from 6 to 15 days are required for curly-top virus to pass a graft union in Turkish tobacco. Under normal conditions the plant grows considerably in this period. Thus, the question arose as to whether the variation in symptoms observed might not be due to age or size of plant at the time of invasion by the virus. The daily records of time of appearance and severity of symptoms from 2 experiments in which healthy Turkish tobacco plants were grafted with cions from recovered plants furnished a partial answer to this question. These records are summarized in table 4. There was but little correlation between severity of symptoms and time when the symptoms first appeared.

It is of interest that when days after grafting were plotted against the number of plants showing symptoms the data followed an S-shaped curve. Part of the variation in time of appearance of symptoms was undoubtedly due to the variation in time required for virus to pass the graft union; part was probably due to the variation in rate of upward movement and distance traveled once the union was passed.

TABLE 4.—*Time required for symptoms to appear in tobacco plants after grafting with cions from recovered plants*[a]

Days after grafting when symptoms first appeared	10	11	12	13	14	15	16	17	19	20	24	30 or more
Symptom type	+++	++++	++	++++	+	+++	++	+	+	+++	+	+
	+++	+		+++	+	++++	++++	+	++		+	+++
	+++	++			+	+	+	++	++		+	+
					++	+	++++	+	+			+
					++			+	+++			+
					++++			+++	++++			+++
								+	+			++
									+			

[a] For explanation of symbols, see footnote to table 1.

Insect Transmission to Large Plants. Additional evidence that variation in symptoms was not due to age or size of plant at time of infection was obtained by exposing large Turkish tobacco plants to viruliferous insects. Eighteen large plants, 6 of which were trimmed as though for cleft-grafting, were exposed. Seven of the untopped plants and 3 of the topped ones came down with severe symptoms, while 5 of the former and 3 of the latter failed to become infected at all.

Cleft-grafting Old Plants. Still further evidence that age of plant has little influence on severity of symptoms was obtained by cleft-grafting 12 old Turkish tobacco plants at a point between $2\frac{1}{2}$ and 3 feet above the soil level. As usual, the symptoms varied with the individual plants. They were severe on 6, moderately severe on 3, moderate on 1, and mild on 2 plants.

Effect of Forcing Grafted Plants into Rapid Growth. It was thought possible that there might be a difference between symptoms produced by rapid-growing and slow-growing plants after grafting with cions from

TABLE 5.—*Symptoms produced in plants cleft-grafted with cions from recovered plants and subsequently forced into rapid growth or not forced*[a]

	Plant No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Test 1												
Forced	+	++++	++++	+	−	++++	++++	++++	++++	+++	++	+
Not forced	+	+++	++++	−	++++	+	++++	++++	++++	++++	++++	−
Test 2												
Forced	++	+	++++	++	++++	++++	+	+++	++	+	+	−
Not forced	−	−	+	+	+++	−	+	++++	−	+	+	−

[a] For explanation of symbols, see footnote to table 1.

recovered plants. To test this, stock plants were grown in 6-inch pots to a height of about 3 feet; half were then repotted in 8-inch pots, while the remainder were left in 6-inch pots. All of the plants were cut back to a point about 12 inches above the soil level, then cleft-grafted with cions from recovered tobacco plants, and held for observation for 2 months. The symptoms produced are recorded in table 5. There was no consistent difference between the symptoms produced on the two groups of test plants. It could not be concluded that variation in symptoms of individual plants was correlated with variation in growth rate.

Symptoms Produced when Viruliferous Insects Were Fed on Various Portions of Turkish Tobacco Plants. As mentioned previously, when

TABLE 6.—*Symptoms produced on plants exposed to viruliferous insects and on plants inoculated by grafting*[a]

Test		Plant No.					
		1	2	3	4	5	6
1	Insects on tip leaves	−	++++	++++	++++	++++	−
	Insects on single leaf	++++	−	+	++++	−	++++
	Insects on stem	−	−	−	−	−	−
	Grafted on stem	++	+	+	−	+	−
2	Insects on tip leaves	++++	++++	++++	++++	++++	++++
	Insects on single leaf	−	−	−	−	+++	+
	Insects on stem	−	−	+	−	+	−
	Grafted	+	−	−	+	+	++
3	Insects on tip leaves	−	−	++++	−	++++	++++
	Insects on single leaf	+++	−	++++	−	−	−
	Insects on stem	−	−	++	−	++	−
	Grafted	−	−	−	−	−	−

[a] For explanation of symbols, see footnotes to table 1.

tobacco plants were grafted with cions from diseased or recovered plants, 6 days or more were required for virus to pass from the cion into the stock. During this period the grafted plants continued to grow and the terminal bud got further and further away from the graft union. Thus, in order to cause symptoms, it was necessary for the virus to move upward several inches at least. On the other hand, when transmission was by means of the insect vector, the virus might be introduced into tissues very near the growing point and thus might infect the growing point quickly. It has been shown by Bennett (2) that, while downward movement of the curly-top virus is rapid in tobacco plants, upward movement is slow. To compare the effects of insect transmission and graft transmission, it was necessary to keep these facts in mind. To draw conclusions from such a comparison, it was essential that the conditions under which the two types of transmissions were employed should be as nearly alike as possible. This could be done by delaying the insect feeding for at least the minimum period required for virus to cross the graft union and by confining the insects to a portion of the plant at the same relative distance from the terminal bud as the graft.

A number of experiments were conducted in which this was done. In each experiment the plants were divided into groups. Viruliferous insects were allowed to feed in the usual manner on one group; they were confined to a single leaf 3 or 4 inches below the tip in a second group; they were confined to a 2-inch portion of the stem 3 or 4 inches below the terminal bud in a third group. Another lot of plants was grafted laterally with cions from plants that had recovered from curly top. The symptoms induced in the experiments are summarized in table 6. Plants exposed to viruliferous

FIG. 1. Turkish tobacco plants infected with curly-top virus. The plant on the left, showing moderate symptoms, was infected by allowing viruliferous insects to feed on a 2-inch section of stem 6 inches above the soil level and about 3 inches below the terminal bud 39 days before the picture was taken. The plant on the right, showing severe symptoms, was infected by allowing viruliferous insects to feed on its apical leaves 39 days before the photograph was taken. (Photograph by J. A. Carlile.)

insects in the usual manner either developed severe symptoms or failed to become infected at all. Those inoculated by grafting showed the usual variation in symptom expression. Three of the plants inoculated by insects feeding on a single leaf or a 2-inch portion of the stem showed mild symptoms only, 2 showed moderate, 2 moderately severe, and 4 severe symptoms, while 24 either failed to become infected or failed to allow virus to move to the tip before the plants had matured. The results obtained by confining

the insects to portions of the plant well below the terminal bud were, therefore, comparable to those obtained with the grafting process (Fig. 1). The conclusion to be drawn was obvious. If the insects were capable of transmitting either a mild or a severe disease, depending upon the portion of the plant upon which they fed, then it could hardly be reasoned that the insects transmitted only the virus, while the grafting process transmitted both virus and protective substances. That such substances may be produced in tobacco plants infected with curly-top virus is certainly a possibility, but the evidence available is not sufficient to justify the conclusion that they exist.

Experiments with Tobacco Cions on Tomato Stocks

Failure to demonstrate antibodies in tobacco plants grafted with tobacco cions led to a test of Wallace's (21) conclusion that tomato plants could be passively immunized from curly top by grafting them with cions from tobacco plants that had recovered from the disease. Of 44 tomato plants grafted either terminally or laterally with cions from recovered tobacco plants, 29 became severely diseased. In many of these the symptoms were much delayed; a few plants first showed symptoms when they had nearly reached maturity. In some, however, the plants were attacked early and promptly died. The remaining plants, 15 in number, failed to show symptoms before they matured. The results with the grafted plants were comparable to those obtained in plants infected by means of the insect vector and in those grafted with cions from diseased tomatoes. Of 27 plants exposed to viruliferous insects, 10 developed a severe disease and 17 apparently escaped infection. Of 34 plants grafted with cions from diseased tomatoes, 12 became severely diseased and 22 showed no symptoms whatever. All the tomato cions died but apparently not before some of them made a union with the stock and transmitted the virus.

It is of interest in this connection that Shapovalov (15) likewise found difficulty in getting diseased tomato cions to form a union with healthy tomato stock, and he was able to transmit the virus to only one of 13 plants approach-grafted to severely diseased plants. However, he did obtain transmission across the graft union in 67 per cent of the cases when the graft was made prior to or simultaneously with exposure of the cion to viruliferous insects.

The reason for the discrepancy between the writer's results with tomato and those of Wallace is not clear. It is possible that growing conditions or other environmental influences may have an effect on immunization of tomato plants by grafting with cions from recovered tobacco plants. It is obvious from the above results that, of the 44 grafted plants, 29 not only were not immunized but actually succumbed to the virus introduced by grafting.

The results of these experiments with tomato stocks, like those of experiments involving tobacco stocks, failed to bring evidence tending to justify an assumption that antibodies were produced by infected plants.

DISCUSSION

The conclusion of Wallace (20) that tobacco plants acquire an immunity from the curly-top disease is supported by results obtained in this study: Turkish tobacco plants suffering from an acute attack of curly top invariably recovered from the disease but did not lose the virus. They did not again develop severe symptoms when pruned back or reproduced by cuttings and forced into rapid growth. Reinoculation of recovered plants either by means of insects or by grafting did not induce further symptoms in them.

On the other hand, the work here reported does not support Wallace's hypothesis that protective substances are transmitted from recovered to healthy plants by grafting. Indeed, no direct evidence was obtained that such substances are produced at all by affected plants. The fact that either a mild or a severe type of disease resulted from insect transmission, depending upon the portion of the plant on which the insect fed, clearly indicates that the mild symptoms induced in some plants by grafting with cions of recovered plants was due to the point of inoculation, not necessarily nor probably to antibodies that might have been carried by the cion.

What then is the true explanation for the production of mild symptoms in some plants and severe symptoms in others after grafting with cions from recovered plants? It is possible to derive an hypothesis that fits the known facts much better than the antibody hypothesis. When the insect vector feeds on tissues near the growing point, it injects a relatively large quantity of virus into or close to those parts of the plant that are subject to malformation; that is, into immature or embryonic tissue. The virus has only a short distance to travel and it arrives in quantity before the tissues have an opportunity to set up a defense against it. On the other hand, when the virus is introduced into a plant some distance below the growing point, either by means of the vector or by grafting, it moves upward slowly and apparently with difficulty (2). Moreover, this movement is erratic, being more rapid in some plants than in others. If only minute quantities of virus reach the immature tissues in the terminal or lateral buds, these tissues are able to set up a defense against it and thus be partly or completely protected against the effects of additional quantities of virus to which they may be subjected. The completeness of the defense might depend upon the original dose of virus and the rapidity with which this dose is increased. If the original dose is large and quickly followed by increase, the tissues may be able only to overcome partly its effects. But, if the original amount to which the embryonic tissues are subjected is small and increase is slow, then they may suffer only slightly from its effects.

The variation in symptoms produced by grafting with cions from recovered plants would thus be explained on the basis of variation in the quantity of virus that moved from the union to growing points of the plant. This variation in the quantity of virus that moved would explain why one axillary shoot on a terminally grafted plant became severely diseased and another on the same plant showed only mild or moderate symptoms.

If it be assumed that tobacco plants recovered from curly top contain less virus than those that are severely diseased, as in the case of tobacco-ring-spot disease (9), then it is possible to explain the tendency for grafting with cions from recently diseased plants to induce, on the average, more severe symptoms than cions from recovered plants. The lower virus content in the latter case would increase the probability that the growing point of the grafted plant would receive a smaller quantity of virus. Hence, there would be a greater probability that such plants would show less severe symptoms than those grafted with cions from recently diseased plants.

There are few, if any, facts available on which to base a conjecture of the nature of the defense mechanism. By analogy with what is known about the nature of acquired immunity from virus disease in animals, the most obvious suggestion is that antibodies are involved. However, the mechanism may be of an entirely different nature, and the production of severe symptoms in plants exposed, through grafting, to any antibodies that might pass a graft union tends, if anything, to indicate that it is.

The correlation between the route of inoculation and the severity of symptoms produced in curly top of tobacco is analogous to the correlation between portal of entry of virus and severity of disease in many animal virus diseases. A few such cases will suffice for illustrative purposes. Theiler (17) was able to infect mice with yellow-fever virus by inoculation directly into the brain. Virus isolated from the brains of infected mice was extremely virulent for mice when inoculated intracerebrally and usually resulted in death. The same virus inoculated subcutaneously, intradermally, intramuscularly, or intraperitoneally seldom resulted in illness or death but often produced an immunity from a subsequent intracerebral inoculation. The mouse-passage strain of virus was low in virulence for monkeys. Sellards (12) reported that, when this strain of virus was introduced intraperitoneally into monkeys, it caused no ill effects but immunized the monkeys against a typical strain of yellow-fever virus. On the contrary, inoculation of the same virus into the brain of monkeys resulted in paralysis and death within a few days. It was shown by TenBroeck and Merrill (16) that rabbits usually were killed by intracerebral inoculation with the eastern strain of equine encephalomyelitis virus, but that rabbits given intracutaneous or subcutaneous injections of the same virus usually recovered and became immune from intracerebral inoculation. According to Rivers and Schwentker (11), large amounts of psittacosis virus could be introduced intravenously and intramuscularly into monkeys without causing serious ill effects, but only small amounts of the same virus introduced intratracheally were required to produce psittacosis pneumonia. It was reported by Traub (18) that intracerebral inoculation of mice with the virus of lymphocytic choriomeningitis usually resulted in convulsions followed by death, but that inoculation with the same virus by the intranasal, intraperitoneal, or intracutaneous route not only failed to cause death but resulted in an immunity often associated with persistence of virus. King

(5) has stated that the effect on mice of a fixed strain of equine encephalomyelitis virus—that is, one passed serially through the brains of pigeons—was dependent upon the route of inoculation. He listed these routes according to their effectiveness in the following descending order: intracerebral, intraocular or intranasal, intravenous, intraperitoneal, intramuscular, subcutaneous.

Because of the difference in the structure and physiology of plants and animals, this apparent similarity in relative effectiveness of different modes of inoculation between these animal virus diseases and curly top in tobacco may be superficial and of no particular significance. It is, however, suggestive of some fundamental characteristic or mechanism common to all these virus diseases, particularly when it is remembered that curly-top virus is confined almost entirely, if not entirely, to the phloem of diseased plants (1) and that all the animal viruses mentioned are specialized for some particular tissue or tissues within the animal body.

SUMMARY

Grafting healthy Turkish tobacco plants with cions from tobacco plants recently infected with sugar-beet curly-top virus resulted sometimes in severe symptoms, sometimes in mild symptoms, and sometimes in symptoms of intermediate severity. Similar results were obtained when tobacco plants were grafted with cions from tobacco plants that had recovered from curly top. On the average, cions from recently diseased plants caused somewhat more severe symptoms than cions from recovered plants, but the difference was not consistent.

Grafting healthy tomato plants with cions from recovered tobacco plants resulted in production of severe symptoms, usually ending in death, or in complete absence of symptoms. In the latter case the virus either failed to pass the graft union or failed to reach the growing points of the grafted plant.

The variation in symptoms occurring in tobacco plants grafted with cions from recovered plants could not be accounted for on the basis of variation in plants or plant position from which cions were taken, of interaction of virus strains, of age of test plants at time of infection, or of growth rate of inoculated plants. There was but little correlation between severity and time after grafting when the symptoms first appeared.

The severity of symptoms induced in Turkish tobacco plants by sugar-beet curly-top virus after infection by means of the insect vector was dependent in part upon the portion of the plant on which the vector fed. Severe symptoms were invariably produced when the vector fed on tissues near the growing point, but frequently only mild or moderate symptoms resulted when the insect was confined to single leaves or portions of stem well below the growing point. Transmission of the virus to old tissues by the insect vector was thus comparable to transmission by grafting with cions from

recently diseased or recovered tobacco plants. These results leave doubt that any protective substances are transmitted through the graft union.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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COMPARISON OF CERTAIN MERCURY AND NON-METALLIC DUSTS FOR CORN SEED TREATMENT[1]

P. E. HOPPE[2]

(Accepted for publication October 7, 1942)

The limitations on the civilian use of mercury necessitates an evaluation of non-metallic seed treatment dusts as possible substitutes for the mercury compounds in common use. The writer reports herein the results of laboratory and field experiments comparing the relative effectiveness on corn of the following seed disinfectants: Spergon (tetrachloro-para-benzoquinone), Thiosan,[3] (50 per cent tetramethyl thiuramdisulphide), New Improved Semesan Jr. (ethyl mercury phosphate, 1 per cent), and Barbak D (mercuric phenyl cyanamide, not less than 6 per cent). Barbak D differs from Barbak C (the corn disinfectant heretofore put out by the American Cyanamid and Chemical Corporation) in that it contains 2 per cent less of the mercury compound than the latter.

LABORATORY EXPERIMENT

This experiment was designed to compare the seed disinfectants with respect to their fungicidal effects on *Diplodia zeae* (Schw.) Lév., one of the commonest and most virulent of the corn seedling blight fungi. In these tests diplodia-rotted kernels were surface-sterilized by immersing for 10 minutes in 0.46 per cent sodium hypochlorite solution, were dried and then dusted by dipping the kernels while held in forceps into the seed disinfectant. Excess dust was removed from the kernels. The kernels then were plated on slightly acidified potato dextrose agar in Petri dishes, one kernel per plate. The plates were kept at room temperatures and observations were recorded on the inhibitory effects of the various dusts as indicated by the amount and rate of growth of *D. zeae* from the corn kernels.

RESULTS

All dust treatments retarded growth of the fungus and some of them often inhibited completely the growth of the fungus in the plates. Ranked in the order of their efficiency in retarding or inhibiting the fungus, the dusts were, New Improved Semesan Jr., Thiosan, Barbak D, and Spergon. The differences between the first 3 dusts mentioned were slight but Spergon was definitely inferior (Fig. 1). The experiment was repeated many times with practically identical results. In all these tests Spergon invariably was

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2] Associate Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] In experiments conducted since this manuscript was submitted, the writer has found the Dubay nonmetallic products, Thiosan and Arasan, essentially the same as to fungicide value and in ability to control *Diplodia* and *Gibberella* in seedborne infections.

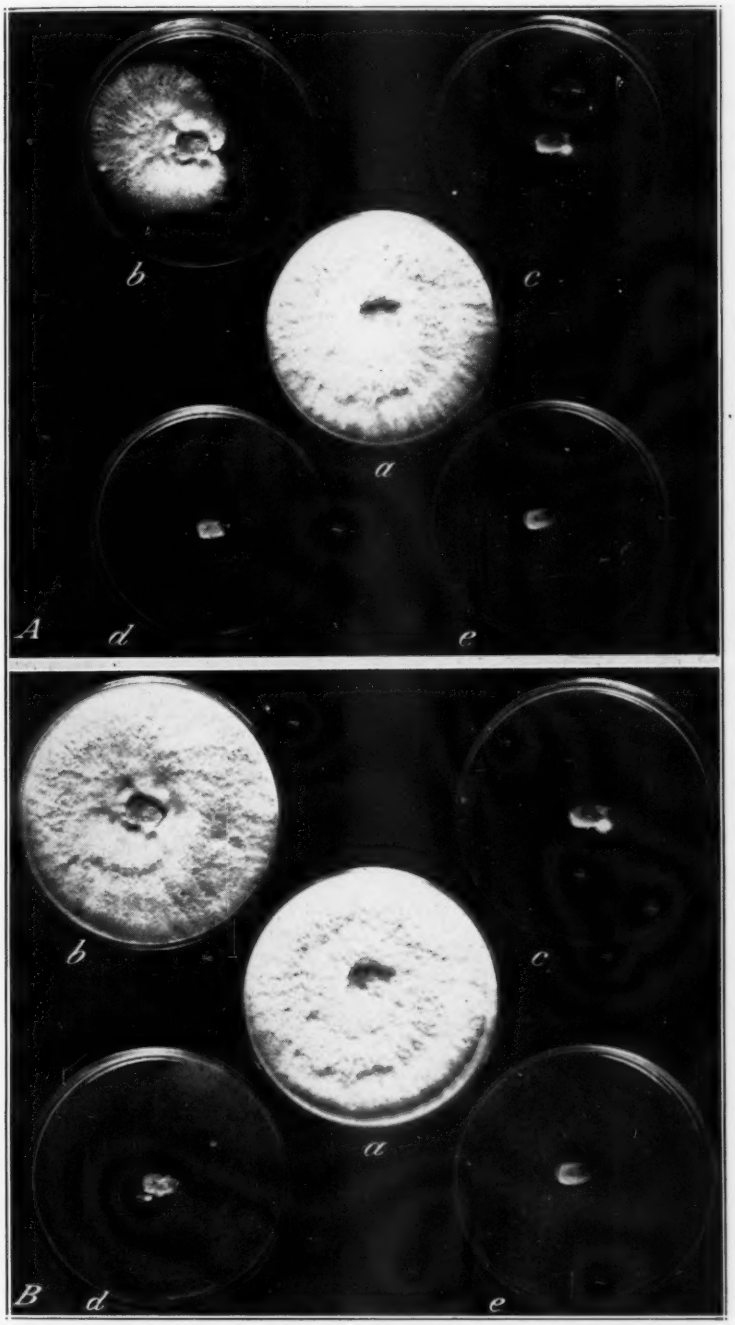

FIG. 1. Growth of *Diplodia zeae* from infected corn kernels given various seed treatments. *A*, 5 days after plating. *B*, Same plates 8 days after plating. The kernels were surface sterilized and then treated with the following seed disinfectants: *a*, control, not treated; *b*, *c*, *d*, *e*, treated with Spergon, Barbak D, Thiosan, and New Improved Semesan Jr., respectively.

conspicuous in its relative inability to inhibit the growth of *Diplodia zeae* from the rotted kernels.

A majority of the kernels in all the treatments eventually developed *Diplodia zeae*. The differences between treatments were mainly in degree of retardation. Throughout the experiment, which continued 10 days, the percentage of kernels that remained free from the fungus was higher in those treated with New Improved Semesan Jr. than in those treated with any of the other dusts.

FIELD EXPERIMENT

Both diplodia-infected hybrid seed and nearly-disease-free inbred seed of dent corn were used in a field experiment. Unfortunately, the Bayer-Semesan Company's non-mercury product, "DuBay" 1205–FF (Thiosan), was not included in the field trial since the writer had none in his possession at the time of planting.

A limited amount of seed that was 100 per cent infected with *Diplodia zeae*, with the degree of infection so restricted that the kernels were germin-

TABLE 1.—*Stands from diplodia-infected and from nearly disease-free seed lots of corn following seed treatment with Spergon, Barbak D, and New Improved Semesan Jr.*

Type of seed	Seed treated with			
	Control not treated	Spergon	Barbak D	New Imp. Semesan
	Per cent	*Per cent*	*Per cent*	*Per cent*
Diplodia-infected hybrid	24.4	40.0	77.8	91.1
71 nearly disease-free inbreds	79.8	85.4	82.1	84.5

able, provided excellent material with which to compare the dusts for ability to control seedling blight from seed-borne infection. This seed was obtained by a very careful selection of kernels from hybrid ears that had been artificially inoculated with the fungus the previous season too late for complete ear rot development. The limited amount of the diplodia-infected seed available permitted only 45 kernels for each of the three seed treatments and the control. Each of these lots was planted in a 5-foot row.

The nearly disease-free seed consisted of 71 inbred lines of corn, obtained, for the most part, from the seed stocks of the Wisconsin Agricultural Experiment Station. These 71 inbreds were each planted 50 kernels per 5-foot row, in 2 replications, making 100 kernels for each inbred and a grand total of 7100 kernels for the inbreds for each treatment and for the controls.

The experiment was planted April 18, 1942, at Madison, Wisconsin, almost a month earlier than the usual corn planting time for this location. Planting was followed by a week of abnormally warm weather after which temperatures dropped and conditions became more favorable for seedling blight development. On the whole, conditions were not so favorable for the disease as might be expected some years.

RESULTS

Final notes were taken the first week in June when most of the seedlings had passed the 4-leaf stage of development. The data for stands for both the diplodia-infected and the nearly disease-free seed are given in table 1.

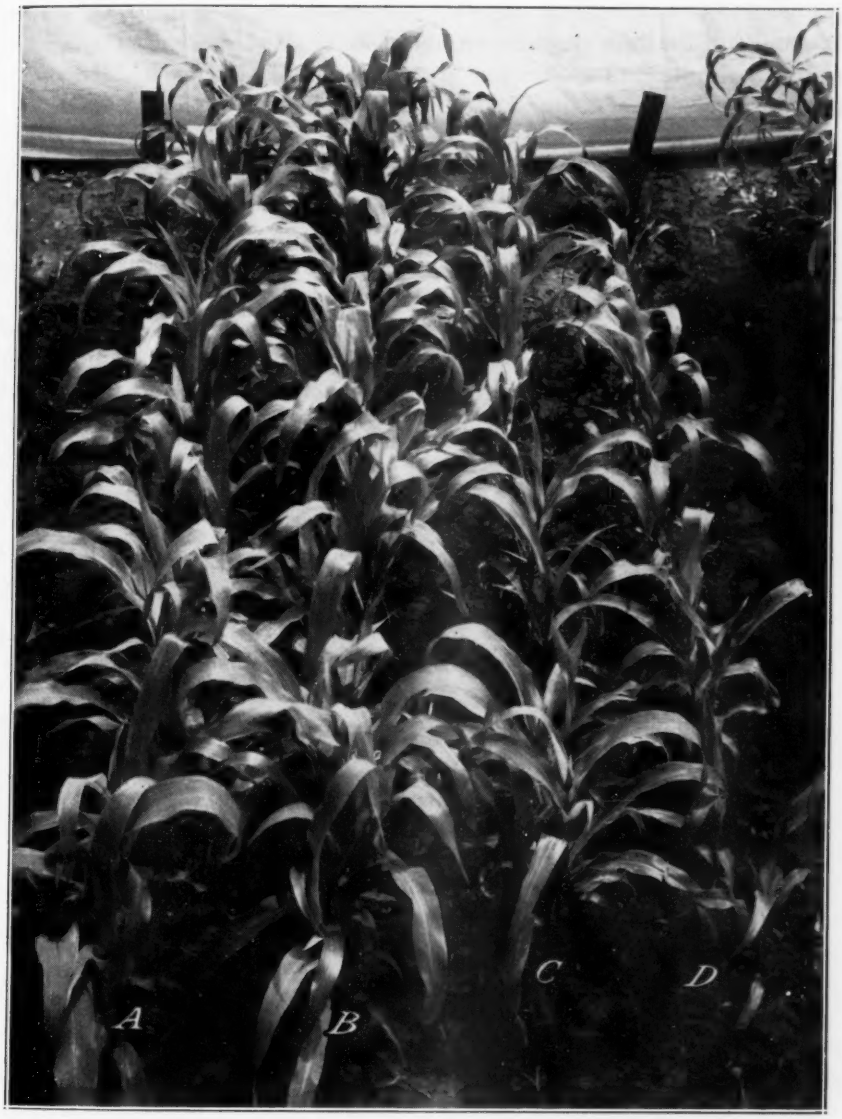

FIG. 2. Differences in stand and vigor of corn seedlings following seed treatment of diplodia-infected seed with different dust disinfectants. *A*, Barbak D. *B*, New Improved Semesan Jr. *C*, Spergon. *D*, Control. (Data in Table 1.)

Spergon nearly doubled the stand of the diplodia-infected control, but proved very inferior to the mercury dusts in controlling seed-borne infec-

tion. The differences between Spergon and the mercury dusts were actually greater than is indicated by the differences in stand. Most of the plants in the Spergon treated row were stunted and were found to have badly diseased mesocotyls. The plants in the rows treated with Barbak and New Improved Semesan Jr. were larger and relatively free from disease (Fig. 2).

All treatments of the nearly disease-free seed resulted in slight increases in stand, but the differences among the three kinds of dusts are small and of doubtful significance. Spergon appears to have been as effective as the mercury dusts in protecting the seed from soil infection.

DISCUSSION OF RESULTS

One cannot generalize broadly on the subject of seed treatments from the results of experiments as limited in scope as those described above. The data and observations are reported in the hope they will be useful in supplementing the results of experiments by other workers.

The results obtained indicate promise for non-metallic dusts as possible substitutes for the mercury compounds. Those with Spergon indicate limitations in its usefulness in controlling seed-borne infection. It may have value, however, as a protectant dust for sound seed planted under unfavorable conditions. As was mentioned previously, it is very unfortunate that the Bayer-Semesan Company's non-metallic dust, "DuBay" 1205–FF (Thiosan), was not included in the field experiment. This dust was far superior to Spergon and about equal to the mercury dusts in the laboratory tests in inhibiting *Diplodia zeae*.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

PYTHIUM ULTIMUM AND THE DAMPING-OFF OF COTTON SEEDLINGS[1]

C. H. ARNDT

(Accepted for publication October 3, 1942)

Pythium ultimum Trow[2] has been repeatedly isolated from diseased cotton seedlings in a field of well-drained, light sandy-loam soil at Clemson, South Carolina. This fungus also has been isolated from a small number of diseased seedlings in other areas during periods of cool, rainy weather by the writer and by Weindling, *et al.* (5). These observations on the association of weather conditions with the isolation of this fungus suggested the study of the relation of soil temperature to infection by this fungus as reported in this paper.

METHODS

Cultures of the fungus were obtained from seedlings with small lesions by washing them with sterile water and then placing the diseased portion of the hypocotyls on raisin-oatmeal agar, adjusted to pH 5 with KH_2PO_4. Bacteria were eliminated from the cultures by making several successive hyphal-tip transfers from rapidly growing mycelia. Three isolates were used in the experiments. As they all showed a similar pathogenicity, no distinction between them will be made in the experimental data.

The effect of soil temperature on the infection of cotton seedlings by this fungus was determined by growing the seedlings at temperatures of 18, 21, 24, 27, and 30 degrees C. in soil-temperature tanks of the University of Wisconsin type. In each of the 25×50 cm. metal cylinders were placed 9 kg. of a steamed, light sandy-loam soil, which had a water-holding capacity of 36 per cent. A holard of 60 per cent was maintained by adding water as needed. Before planting, a one-eighth sector of 4-day-old culture of the fungus grown in a 9-cm. Petri dish on raisin-oatmeal agar was mixed into the upper portion of the soil of each cylinder. A like amount of sterile culture medium was added similarly to the cylinders in which the controls were grown.

Acid-delinted seed of a strain of Cleveland Big Boll cotton (Marett's 5–5) were used. To assure that only viable, disease-free seedlings were used, the seeds were immersed for 2 minutes in $\frac{1}{2}$ per cent $HgCl_2$ in 50 per cent ethanol, then rinsed with sterile water, and germinated at 30° C. on sterilized, moistened filter paper in Petri dishes, until the radicles had emerged 2–3 cm. The germinated seeds were placed, radicle end down, in 21 regularly spaced holes, 1×4 cm., that had been punched into the soil. The holes were then filled with soil and the surface was covered with 2 medium-weight discs of asphalt-impregnated roofing paper to facilitate

[1] Technical contribution no. 88 from the South Carolina Agricultural Experiment Station.

[2] Acknowledgment is made to Dr. Charles Drechsler for the verification of this determination.

maintenance of constant soil moisture and temperature. The discs were removed from the cylinders as soon as the cotyledons began to emerge, which varied with the temperature from 40 to 160 hours (Table 1) after planting. After the removal of the paper discs, the soil temperature at a depth of 2 cm. did not vary more than $\frac{1}{2}°$ from the mean temperature. Nearer the surface the variation was greater depending upon the differences between the soil and air temperatures and the intensity of the sunlight. All percentages in the tables, unless otherwise noted, are based on the planting of 21 seeds in each of 6 cylinders. The weights of the tops, the roots having been cut off at the base of the hypocotyl, were used to determine the relative growth of the seedlings.

EXPERIMENTAL RESULTS

The results for one experiment in which the plants were grown in the greenhouse from March 21 to April 24 are given in table 1. During this

TABLE 1.—*The effect of soil temperature on the emergence and damping-off of cotton seedlings in soil inoculated with Pythium ultimum*

Soil temperature	Total emergence		Seedlings killed first 14 days	Seedlings after 30 days		
	Relative to controls	Time required		Alive	Healthy	Mean weights relative to controls
°C.	*Per cent*	*Hours*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
30	97[a]	40	6	94	80	97
27	87	44	45	55	10	56
24	77	60	60	40	10	55
21	68	110	83	0	0	.....
18	38	160	90	0	0	.....
15–30[b]	70	90	65	63	0	78
Greenhouse[c]	50	160	85	30	0	31

[a] Temperature 30° for 12 hours during the day and 15° at night.
[b] Plants on greenhouse bench. Containers similar to those used in constant temperature tanks.
[c] Estimated from plants removed after 14 days. Few lesions evident on the 30th day.

period the daily minimum range of air temperatures was 10–18°, with a mean of 16°; and the daily maximum range 20–37°, with a mean of 30°. In this experiment at 30, 27, 24, 21, and 18 degrees C., the seedling emergences were, respectively, 3, 13, 23, 32, and 62 per cent less than in the controls at the same temperatures, in which the emergences ranged from 93 to 100 per cent. When the alternating periods of 12 hours each at 15° and 30° were used, the reduction in emergence, as shown in table 1, approximated that at a constant temperature of 21°; while in the cylinders on the greenhouse bench, the emergence was slightly lower than at 21°. Fourteen days after planting, the seedlings in the cylinders at 27° and 30° were reduced to 10 to prevent crowding. These seedlings were removed by random selection according to a prearranged plan to avoid a possible bias in the removal of diseased and healthy seedlings. There were lesions on 20 per cent of these seedlings grown at 30°, and on 90 per cent of those grown at 27°. No seed-

lings were removed from the cylinders at the lower temperatures, as the number and size of plants was not sufficient to cause crowding.

At 30° 6 per cent of the seedlings that emerged were killed within the first week; then, there was no further evidence of injury by the fungus. At this temperature 20 per cent of the hypocotyls of the seedlings removed at the end of 14 days showed small superficial lesions. The injury was not sufficient to cause a stunting similar to that occurring at all lower temperatures. The degree of stunting of the seedlings is indicated in table 1 by the relative green weights of the seedlings in the controls and those in the inoculated cylinders.

At 27° and the lower temperatures the fungus invaded the stele of the hypocotyl and infected the roots, in contrast to the superficial lesions produced at 30°. All killing of the seedlings at 24° and 27° occurred within the first 14 days after planting, and all plants surviving after that period

TABLE 2.—*Injury to cotton seedlings by Pythium ultimum at soil temperatures of 22°, 27°, 30°; and also at 22° after initial growth periods at 30°*

Soil temperature for successive growth periods			Relative green weight of seedlings grown in non-inoculated soil (controls)	Weight of seedlings in inoculated soil relative to seedlings in controls	Percentage of plants	
Days					With lesions	Killed
1–6	7–12	13–19				
°C.	°C.	°C.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
30	30	30	100	97	30	1
30	30	22	97	81	40	1
30	22	22	94	65	65	5
22	22	22	65	20	100	80
27	27	27	100	63	100	16

were growing normally at the end of the experiment. Only 17 and 10 per cent, respectively, of the plants grown at 21° and 18° were alive after 14 days, and all of these were dead at 30 days after planting. At all temperatures the first evidence of severe injury was a wilting of the seedlings, followed by their death within 2 or 3 days.

After determining the relative pathogenicity of the fungus at the several temperatures, seedlings were grown for 6- and 12-day periods at 30° in inoculated soil and then transferred to 22° to study the interaction of the age of the seedling and soil temperature on infection by this fungus. Three other sets of plants were grown throughout the 19-day period at 22, 27, and 30 degrees C. The atmospheric conditions during the growth of this series were much the same as in preceding series, except for a 3° higher mean minimum temperature. The percentages of seedlings infected and killed at the constant temperatures of 22, 27, and 30 degrees, table 2, were approximately the same as in the first series. In the inoculated soil a 10 per cent greater number of the seedlings grown for an initial period of 12 days at 30° and then transferred to 22° were infected than of those grown continuously at 30°. There was also a 16 per cent greater reduction in the mean weight of

the seedlings. The transfer of the seedlings to 22° after 6 days at 30° caused a further increase of 25 per cent in the number of infected seedlings, and, as might be expected from the lower relative weight, many of the seedlings were stunted. The stunted seedlings were making satisfactory growth on the 19th day and appeared capable of outgrowing the lesions and developing into normal plants. Only 5 per cent of seedlings grown for 6 days at 30° and then lowered to 22° were killed as compared to 80 per cent of those grown continuously at 22°.

In these experiments *Pythium ultimum* produced a soft rot of the cortex of the hypocotyl below the surface of the soil. The affected area was almost colorless to light-brown until the decay of the tissues had become well advanced, when they became darker. The lesions in their initial development tended to involve the entire circumference of the hypocotyl. In this respect they are similar to those produced by *Rhizoctonia solani* and differ from those produced by *Colletotrichum gossypii*, which first appear as elongated sunken areas in the cortex of the hypocotyl (5). Although the hyphae of *P. ultimum* were at first confined to the cortex, at 27° and the lower temperatures, the hyphae invaded the stele causing the wilting and death of the seedlings. This fungus did not seem to attack the roots until after the hypocotyl had been well decayed. In the experiment in which the plants were grown for 30 days, all of the seedlings alive at the end of this growth period were developing normal tissue underneath the lesions and were apparently capable of developing into normal plants. The rapid recovery of the seedlings from lesions produced by this fungus seems to indicate that it is strictly a seedling parasite of cotton. This is also indicated by the fact that no evident stunting of the surviving older plants has been observed in the area of the field in which the fungus had caused severe damping-off. Apparently it is a more transient parasite on cotton than related *Pythium* spp., which parasitize corn (3) and sugar cane (4).

Harter and Whitney (2) have shown that *Pythium ultimum* grows equally well over a temperature range of 23–35° C., the rate of mycelial spread on agar falling off rapidly above 35° and somewhat less so below 23°. Consequently, if the rate of mycelial growth were the only factor involved, the most severe injury to cotton seedlings should have occurred in these experiments at 30° instead of at temperatures below 27°. Similarly, Harter and Whitney (2) found no direct correlation between the relative rate of growth of the fungus at various temperatures and its injury to the sweet potato at the same temperatures. Much the same situation has been found for the *Pythium* species that parasitize the roots of sugar cane and corn. Flor (1) and Rands and Dopp (4) found that the optimum for the growth of these mycelia was close to 30°. Yet Flor (1) found that the damage to corn was greater at lower temperatures and at a high soil moisture content. Injury to corn at 30° was, however, relatively greater than that to cotton seedlings caused by *P. ultimum*.

The widespread distribution and diversity of the hosts of *Pythium ultimum* would seem to indicate that it may be a frequent, although probably

not an economically important, cause of the damping-off of cotton seedlings. Because of the well-known possibility of inhibiting the growth of *Pythium* spp. by most of the fungicides used for the surface sterilization of cotton seedlings before they have been plated on water agar for the determination of infecting fungi (5), it is not unlikely that a different technique might result in a more frequent recovery of this fungus from diseased seedlings.

SUMMARY

Pythium ultimum has been found to be a frequent cause of the damping-off of cotton seedlings in one field in South Carolina when planting has been followed by cool, rainy weather.

When cotton seedlings were grown at soil temperatures of 18, 21, 24, 27, and 30 degrees C. and a soil-moisture content of 60 per cent, this fungus caused little damping-off at 30°, but caused severe damping-off at the lower temperatures. All seedlings were killed at 21° and 18°. The results obtained when the seedlings were grown for 6 days and 12 days at 30° in inoculated soil and were then transferred to 22° indicate that this fungus will cause severe losses from damping-off only if soil conditions are favorable for infection before the seedlings have reached a stage of development comparable to that reached in a growth period of 6 days at 30°.

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4. RANDS, R. D., and E. E. DOPP. Variability in *Pythium arrhenomanes* in relation to root rot of sugar cane and corn. Jour. Agr. Res. [U. S.] **49**: 189–221. 1934.
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PHYTOPATHOLOGICAL NOTES

Freezing Preservation of Fungi and Fungus Spores.—The sporidia of *Gymnosporangium juniperi-virginianae* and the conidia of *Venturia inaequalis* have been used as test fungi in the method for evaluating fungicides for the control of apple scab and cedar-apple rust on potted trees in the greenhouse. Difficulties were encountered in maturing the cedar galls for early season work and keeping them throughout the year after spore discharge was complete in the field. As it was impractical to culture conidia of *Venturia inaequalis* in sufficient quantity for the experiments, the earlier investigations with this fungus were limited to unreliable sources of inoculum from the field. A new technique involving quick-freezing and storage of the galls and conidia at low temperatures has been found to provide a readily

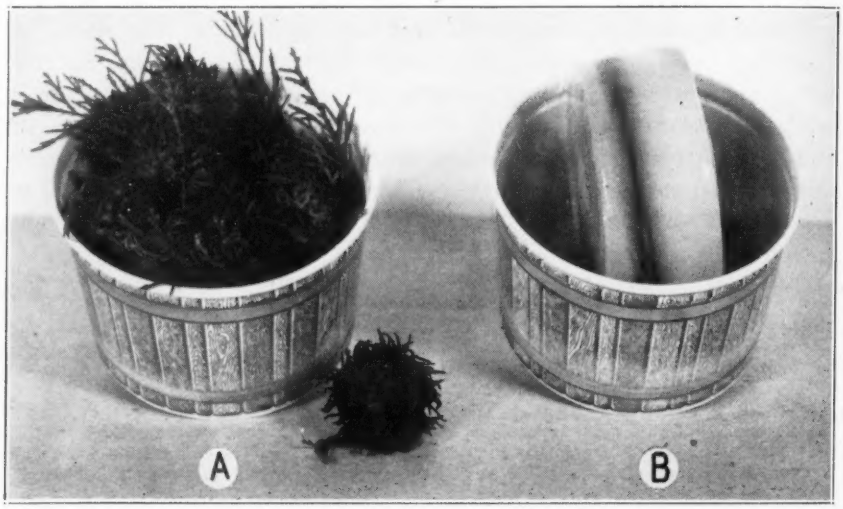

FIG. 1. Fungus material as frozen and stored at −10° C. A. Cedar-apple galls. B. Conidia of *Venturia inaequalis*.

available source of inoculum throughout the year (Fig. 1). It is thought that this procedure would be of interest to other workers interested in the storage of fungi.

STORAGE OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

Cedar galls, three-quarters of an inch and over in diameter, were selected with telial horns approximately one-half inch long when dry, placed in paraffined Dixie cups, and held at a temperature of −10° C. Excessive dehydration over a prolonged period is prevented by placing the cups in vapor-proof metal containers. The galls are removed from cold storage to room temperature about 12 hours prior to placing them in a misty spray in preparation for spore discharge. The germination of teliospores is not impaired by the freezing process. Galls have been first frozen at −40° C., for a short time and then raised to −10° C., but this is not considered essential.

Foliage tests have shown sporidia obtained from the frozen galls as viable as those obtained from galls taken from the field. The teliospores on galls held at – 10° C. for 9 months have functioned normally. It is of special interest that three spore discharges have been obtained from a given gall that had been refrozen after each discharge. Viable sporidia have been obtained 15 months after the first freezing of such galls. Sporidia in water suspension have been stored at – 10° C. and – 40° C., but cannot be held longer than 3 weeks without appreciable loss in viability.

STORAGE OF VENTURIA INAEQUALIS

Concentrated aqueous suspensions of conidia of *Venturia inaequalis*, washed from fresh fruiting lesions on foliage or fruit, are stored at – 10° C. in paraffined Dixie cups. To prevent the spores settling to the bottom of the cups where they would be subjected to dehydration and consequent loss of viability during storage, it is necessary to prepare the cups with a layer of ice frozen in the bottom before the spore suspension is poured. The inoculum is obtained by chipping a portion of ice from the block, defrosting, and diluting to the desired concentration.

Excellent germination of conidia frozen more than 15 months has been obtained. While the frozen conidia may be satisfactory for routine testing on glass slides or on the foliage, they are more readily inhibited by fungicides than fresh-borne conidia. In other words, the slope of the LD–50 line becomes steeper after the conidia have been stored for an appreciable length of time.

The spore suspensions have been frozen at – 40° C. without injury.

This procedure has worked equally well with the conidia of *Sclerotinia fructicola*. Dr. R. F. Suit has had comparable results from freezing the conidia of *Plasmopara viticola*.—J. M. HAMILTON and L. O. WEAVER, N. Y. State Agricultural Experiment Station, Geneva, New York

"Red Ring" of Tomato Stems Caused by an Insect, Cyrtopeltis varians (Dist.), at Charleston, S. C.[1]—During August, 1941, a mirid, *Cyrtopeltis varians* (Dist.),[2] (Fig. 1, C, D), formerly named *Engytatus geniculatus* Reuter, was observed associated with reddish-brown marks (Fig. 1, A) encircling the stems and petioles of tomato plants. This condition was observed again from July to October, 1942, at the Regional Vegetable Breeding Laboratory of the U. S. Department of Agriculture at Charleston, South Carolina. The injury occurred on the upper and youngest branches of the plants, and was most evident late in the picking season. It was observed throughout the plots, occurring on such varieties as Rutgers, Marglobe, Pan America, and Stokesdale.

[1] Contribution No. 28, U. S. Regional Vegetable Breeding Laboratory, Charleston, South Carolina.

[2] Specimens from both 1941 and 1942 collections (E. and P. Q. No. T. C. 3968) were determined by H. G. Barber, Division of Insect Identification, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D. C.

The insect had girdled the stems with punctures approximately 1 mm. deep. Soon after the injury, the marks were barely discernible; later they turned reddish-brown and were usually raised above the normal surface of the stems. Bending the stems or petioles often caused breakage (Fig. 1, B).

This plant bug occurs throughout southern United States to Lower California, Mexico, Central America, Brazil, the West Indies, and the Hawaiian Islands. It has been reported[3] as a tomato pest in Texas and Arizona, where the injuries resembled closely those observed at Charleston. In the former States it was noted also that the stems with younger injuries broke off readily, whereas those with older injuries were quite resistant. This was

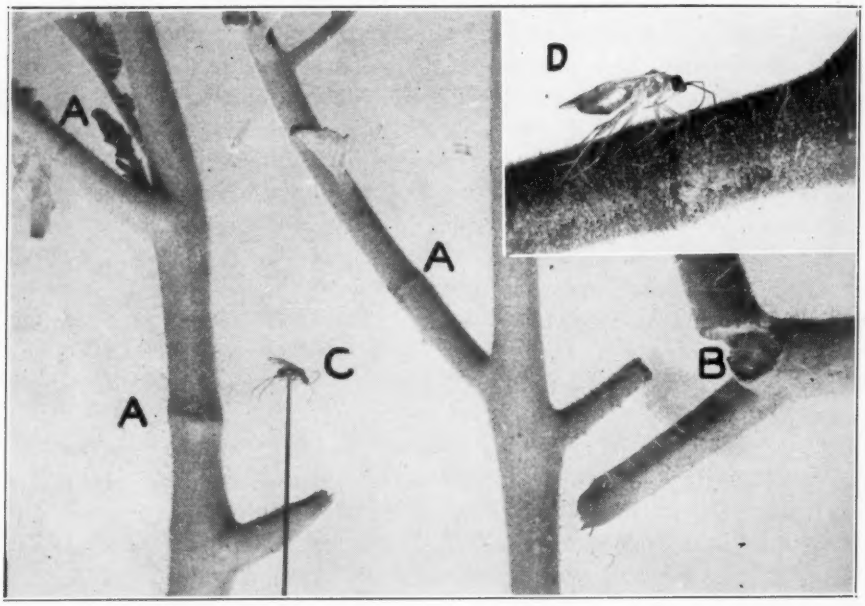

FIG. 1. "Red ring" injury to tomato stems. A. Rings on stems and leaf petioles. B. Stem broken at point of injury. C and D. Insect causing the injury. Note (insert) proboscis extending back from lower part of head. A to C, × 1.5. D, × 5.

also evident at Charleston, suggesting that healing of the wounds strengthened the stems at the points of injury. The insect has been reported[4] as a tomato pest in Georgia and Mississippi, but no descriptions of the injuries were given.

In the Hawaiian Islands the insect may cause tomato-crop failures by sucking the juice from the developing ovaries.[5] It has been observed on the islands of Maui,[6] Oahu,[7] and Kaui[8] in addition to Hawaii. No injuries

[3] Jones, W. W. Tomato vines injured by a Mirid (*Engytatus geniculatus* Reuter). J. Econ. Ent. 24: 327–328. 1931.

[4] Knight, H. H. The plant bugs, or Miridae, of Illinois. Ill. Nat. Hist. Surv. Bull. 22 (1): 53. 1941.

[5] Illingworth, J. F. *Engytatus geniculatus* Reuter—an important pest of tomatoes in Hawaii. Hawaiian Ent. Soc. Proc. 7 (2): 247–248. 1929.

[6] Hawaiian Ent. Soc. Proc. 7 (1): 2. 1928.

[7] *Ibid.*, 7 (1): 11. 1928.

[8] *Ibid.*, 7 (2): 271. 1929.

resembling "red ring" were described in these reports, the principal injuries being to the flower parts.

The Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, has cited an unpublished note made in 1940 by R. E. Campbell and J. Wilcox, located at its Alhambra, California, field laboratory as follows:

> A small bug, *Cyrtopeltis varians* (Dist.) is causing serious damage to tomatoes in Orange County. The immature bugs cluster on the twigs ¼ inch in diameter and less, and their feeding causes a brownish ring which girdles the twig and it usually breaks off.

W. H. White of the above-mentioned Division has made the following comment:

> In a survey performed by Messrs. Campbell and Wilcox it was found that this mirid bug was very numerous in Orange, Los Angeles, and San Diego counties in southern California during 1940. It was generally believed by the growers that the insect was responsible for the poor setting of tomatoes. Although signs of feeding injury of this insect were evident and in many fields the blossoms and newly-set tomatoes had dropped from the plants in large numbers, no definite information was obtained respecting the direct responsibility for the poor setting of the fruit. There was, however, a very evident relation between the number of these insects in a given field and the number of tomato fruits which set.

No serious injury of tomato plants was evident at Charleston, perhaps because the insects attacked only stems and leaves instead of flowers. The fact that the "red rings" might be mistaken for disease symptoms and that the insects might be vectors for some transmissible disease suggested this note on their occurrence.—GEORGE B. REYNARD, Assistant Geneticist, Div. Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Charleston, S. C.

Southern Blight, Corticium rolfsii, of Potato Tubers.—Potato tubers, variety Sebago, infected with *Corticium rolfsii* (Sacc.) Curzi, were observed when harvested in late May, 1942, in a field near Gainesville, Florida. In this field about 1 per cent loss of tubers was recorded.

Infections caused by this fungus on potato plant stems have been observed annually in the Southeastern States and described from Florida by the writer.[1]

Other records[2,3,4,5,6] concerning this disease include various descriptions of infected tubers and tops. Eddins[7] observed the disease in Florida and

[1] Weber, George F. Potato diseases and insects. Fla. Agr. Exp. Stat. Bull. 169: 123–125. 1923.

[2] Edson, H. A., and M. Shapovalov. Parasitism of *Sclerotium rolfsii* on Irish potatoes. Jour. Agr. Res. [U. S.] 23: 41–46. 1923.

[3] Dykstra, T. P. Potato diseases and their control. U. S. Dept. Agr., Farmers' Bull. 1881. 15–16. 1941.

[4] Higgins, B. B. Physiology and parasitism of *Sclerotium rolfsii* Sacc. Phytopath. 17: 417–448. 1927.

[5] Link, G. K. K., and G. B. Ramsey. Market diseases of fruits and vegetables, potatoes. U. S. Dept. Agr. Misc. Pub. 98: 46–48. 1932.

[6] McClintock, J. A. A tuber rot of Irish potatoes. Tenn. Agr. Exp. Stat. Circ. 32: 1930.

[7] Eddins, A. H. Diseases of plants in the United States. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 25: 354. 1941. Suppl. 119: 241. 1939; Suppl. 128: 290. 1940.

recorded resultant losses. The available information describing this tuber disease is so variable and incomplete that a detailed description should be useful.

Suspected tubers, incubated for 24 hours at room temperature, bore the characteristic mycelium of the fungus. Isolated diseased tissue on potato-

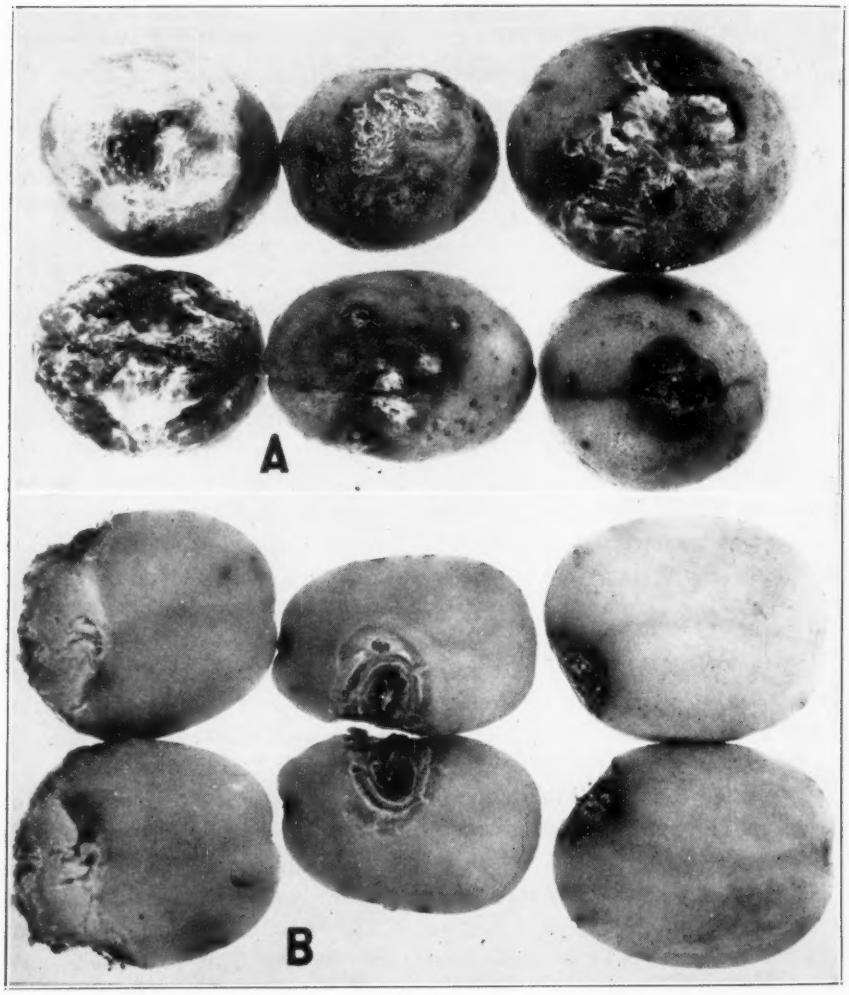

FIG. 1. Potato tubers infected with *Corticium rolfsii*. A. Various stages of development of the disease; note young sclerotia in upper left and center. B. Tubers of second row of A cut and showing internal decay.

dextrose-agar plates characteristically produced the fungus. Mycelium placed in contact with uninjured tubers, and mature sclerotia inserted into tuber wounds produced disease similar to that found in nature. The following symptoms generally characterize the disease (Fig. 1).

Externally the smallest characteristic infection spots were 2 to 3 mm. wide, circular, slightly sunken with brownish margin diffused into surrounding tissue. Area within the margin abruptly paler, with a center speck, apparently a lenticel. These spots isolated, widely scattered or clustered, but not correlated with any part of tubers. As spots enlarged, surface became more noticeably sunken, uniform yellow to tan. Spots about 1 cm. in diameter, gradually darkened as secondary organisms developed on surface. Frequently, tuber cortex surrounding these spots overgrown by thin, more or less reticulate hyphal weft closely applied to surface. As disease advances, portions of the tuber collapse, producing soft areas, usually covered by cortex. Ultimate and extensive development of infection ends in rupture of cortex and usual disclosure of sclerotia, clustered, white to dark-brown, depending on maturity, uniformly spherical and small.

Internally, the small rapidly developing young spots were shallow. As they enlarged and deepened the tuber flesh changed from a wet, firm, more or less transparent condition to an odorless, chalky white, opaque, cheesy consistency, losing its firmness entirely. Invaded portion dried, shrinkage and collapse followed, forming cavities later filled with the white mycelum of the fungus. Sclerotia usually developed in these cavities.

The plot of dry, rather porous, sandy soil, where the diseased tubers were grown, had previously been devoted to vegetable crops and several of them had shown frequent infection from this fungus. Previous observations have shown soil moisture the most important environmental factor affecting the development of the disease. During humid, rainy weather the fungus attacks plants at the soil surface, producing stem girdling and infection on aerial plant parts in contact with the soil. As the soil surface becomes dry the fungus appears to cause infection at successively lower levels. Often plants wilt because of stem girdling when ½ to 1 in. dry mulch exists. In such instances the points of infection are an inch or more below rather than at the surface. Certain deep-set plants frequently cultivated and hilled up during extremely dry seasons have been attacked by the fungus on the main tap root more than 3 inches below the surface. This moisture relationship has been determined by repeated examinations and seasonal observations of *Corticium rolfsii* and its pathogenicity on various wild and cultivated plants in Florida for a score of years. The application of this information here explains, in a general way, the current attacks on potato tubers. The dry season has apparently resulted in the development of the fungus at a deeper level in the vicinity of the tubers, resulting in their infection.—GEORGE F. WEBER, Agricultural Experiment Station, University of Florida, Gainesville, Fla.

A Culture Medium of Maize-meal and Starch Mush to Replace Agar for Growing Fungi.—I have been using a culture medium to partly replace agar in some of my work that may be worth suggesting for trial by others. Supplies of agar are difficult to obtain and a mixture of maize meal and starch was developed to replace routine use of large quantities of agar for some studies. The meal and starch are cheap, obtainable from grocery stores, and not likely to be curtailed by military necessities. In addition the mush medium has produced unique results in certain cultural studies. I have also used it with success as an acidified medium to isolate fungi from infected tissues.

My formulae and methods of preparation are as follows:

1. White maize meal (water-ground), 260 g. or 300 g. if steel-cut; cold tap water, 1000 cc. Mix these, bring to boil, and cook in double boiler or Arnold steam sterilizer for 10 minutes.

2. Corn starch (refined cooking quality), 20 g.; cold tap water 50 cc. Stir together until smooth. Pour into the hot mush just prepared (1), stirring constantly.

3. With spoon, dip about 25 cc. of this final hot-mush mixture into bottoms of Petri dishes and spread quickly and evenly by circular movements with glass rod, "settling" the mush by 2 sharp jolts on the palm of the hand.

4. Filled dishes are then covered and put directly in an autoclave. Pressure is slowly raised to 15 lb., where it is kept for 20 minutes, and then cooled slowly. These plates are allowed to cool overnight before using for culture purposes.

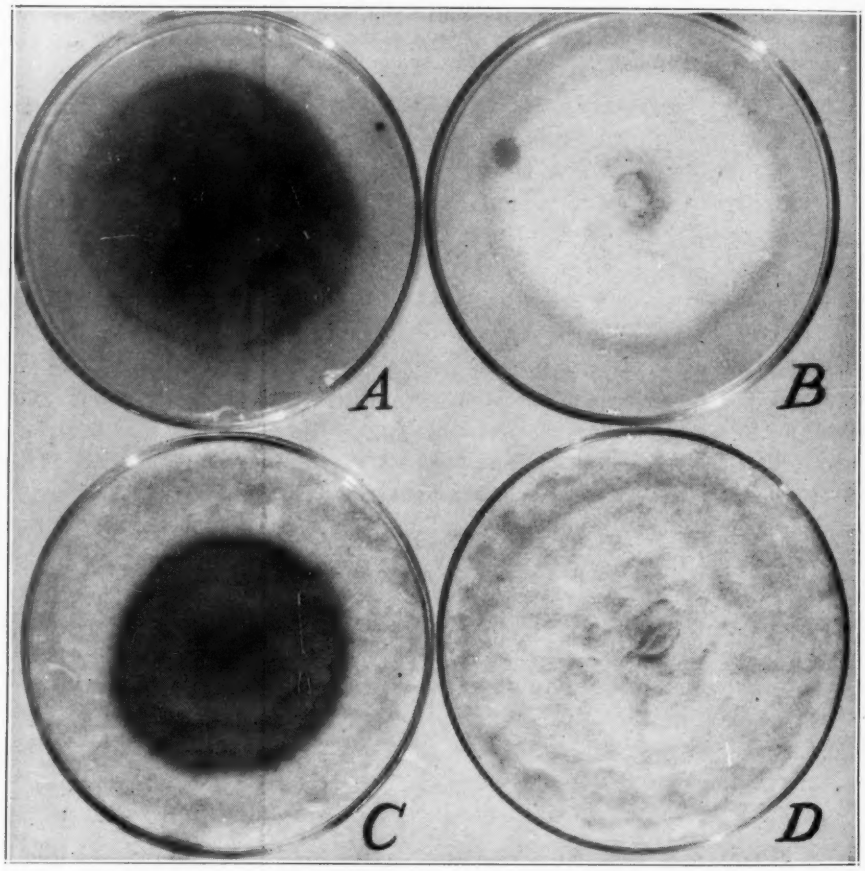

FIG. 1. Comparative growth in parallel cultures: *Alternaria solani*, on agar plate *A* and on maize-meal and starch-mush plate *C*; *Fusarium bulbigenum* var. *lycopersici* on agar plate *B* and on mush plate *D*. Agar is of "differential" formula (Wellman, F. L. Phytopath. 32: 271–287, 1942) known to produce excellent growth of both organisms. Mush-medium formula as given in present paper.

When acidified plates are to be prepared, 4 drops of 25% lactic acid are put on top of the mush before spreading and mixed in each dish at stage 3 of preparation.

Mush, after completing stage 2, may be thinned with about 10 per cent by volume of cold water. This thinner mixture can then be squeezed through a

large-bore funnel into test tubes, where it is slanted by jolting cautiously on the heel of the hand, plugged in the usual manner with cotton, autoclaved in a slanting position, and cooled in a slanting rack.

The mush medium is, of course, opaque, and has other disadvantages when compared with agar. It may not be so neatly prepared as some of the more transparent media. The operator can, however, become adept in its preparation and much culture work can be accomplished without benefit of agar.

Cultures of *Alternaria solani* (Ell. and Martin) Jones and Grout, and *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R. grow very well on the mush medium (Fig. 1) and tests have shown that the cultures retained their pathogenicity. Other species of *Fusarium* and *Alternaria* and species of *Rhizoctonia, Colletotrichum, Penicillium, Aspergillus, Pythium,* as well as contaminating bacteria and yeasts, also have grown readily on the medium.—FREDERICK L. WELLMAN, formerly of Bureau of Plant Industry, now of Office of Foreign Agricultural Relations, U. S. Department of Agriculture, Washington, D. C.

A Reflector Scale for Measuring Growth of Fungi.[1]—Measurement of growth rate in certain microorganisms is a time-consuming process with

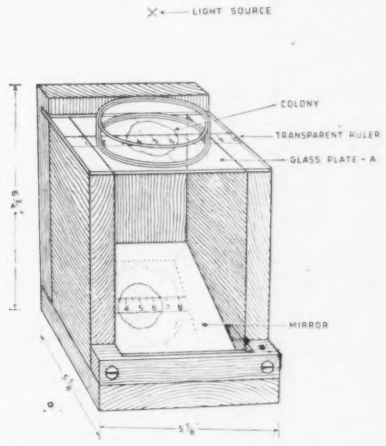

FIG. 1. The reflector scale for measuring fungus cultures.

danger of contamination and formation of secondary colonies when plates are disturbed for periodic measurements. Recently, in connection with a nutritional study of the effect of trace elements on various pathogens, several hundred agar plate cultures of various species of fungi were inoculated to study mycelial growth rate. For a reliable growth curve it was necessary to record the average diameter at 8-hour intervals from 2 measurements made at right angles. For convenience and speed of measurement, and to avoid

[1] Paper No. 2063 in the Scientific Journal Series, Minn. Agr. Exp. Station. Assistance in the preparation of figure 1 was furnished by personnel of the Works Project Administration official project No. 265–1–71–236, Subproject 487.

unnecessary disturbance and scattering of spores over the plate, the following simple apparatus was designed and built (Fig. 1). It is a compact, box-like instrument with a top panel of glass that is scaled in millimeters. A cheap, movable, transparent ruler, inserted just beneath the glass, is satisfactory if the glass is not ruled. A mirror fixed in the base of the frame at a 30° angle reflects whatever is placed on the glass. A desk lamp is placed above the apparatus, so that its light shines through the Petri plates placed right side up on the glass. The mirror image of the fungus culture on the agar is plainly visible and the scale is easily read. The light above the plate facilitates accurate measurements of colonies with extremely fine mycelium and indistinct margins.

The reflector scale may be used for fungus cultures in flasks as well as for cultures in Petri plates.—PHILIP HAMM, J. E. MITCHELL, and DAVID GOTTLIEB, Division of Plant Pathology and Botany, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minn.

Rasp Leaf of Cherry.—In May, 1940, C. W. Neider, State Horticultural Inspector for northern Idaho, sent to the writer a few diseased cherry leaves from a tree in the city of Coeur d'Alene. According to the owner the original sweet-cherry tree had suffered severe winter injury in 1935–36, and sprouts from the roots (mazzard) replaced the tree. No leaf abnormalities had been observed until 1940. The symptoms exhibited by leaves of this tree, and, subsequently, on experimental trees, gave rise to the term *ruffled leaf* by the writer, who at that time had seen no previous mention of this disease or its name in the literature. The characteristics of the disease in Idaho are regarded as identical with what Bodine and Newton designated as rasp leaf[1] and Lott and McLarty[2] called leaf enation. Symptoms are illustrated in figure 1. H. R. McLarty saw the Idaho trees in 1942 and stated that the symptoms apparently were the same as those shown by his material. The similarity in symptoms was noted also by L. C. Cochran who had seen the Colorado rasp leaf condition. In the interests of uniformity and priority the name rasp leaf is preferred.

Transmission trials started in August, 1940, showed by June, 1941, that in all but one or two cases, shoots produced by supposedly diseased buds did not even perpetuate the condition. On 2 mazzard seedlings, however, the stock showed mild symptoms; but, inasmuch as leaf enations may sometimes be found on mazzard seedlings, the proof of transmission was not convincing enough to warrant a statement on such a striking disease. In August, 1941, additional inoculation trials were made involving the use of young Bing trees, Montmorency, and additional mazzard seedling trees and two types of inoculum: (a) buds from the axils of affected leaves and (b) buds from

[1] Bodine, E. W., and J. H. Newton. The rasp leaf of cherry. Phytopath. 32: 333–335. 1942.

[2] Lott, T. B., and H. R. McLarty. Leaf enation. (*In* Handbook of virus diseases of stone fruits in North America, compiled by E. M. Hildebrand, G. H. Berkeley and D. Cation.) Mich. Agr. Exp. Stat. Misc. Pub. 51. 1942.

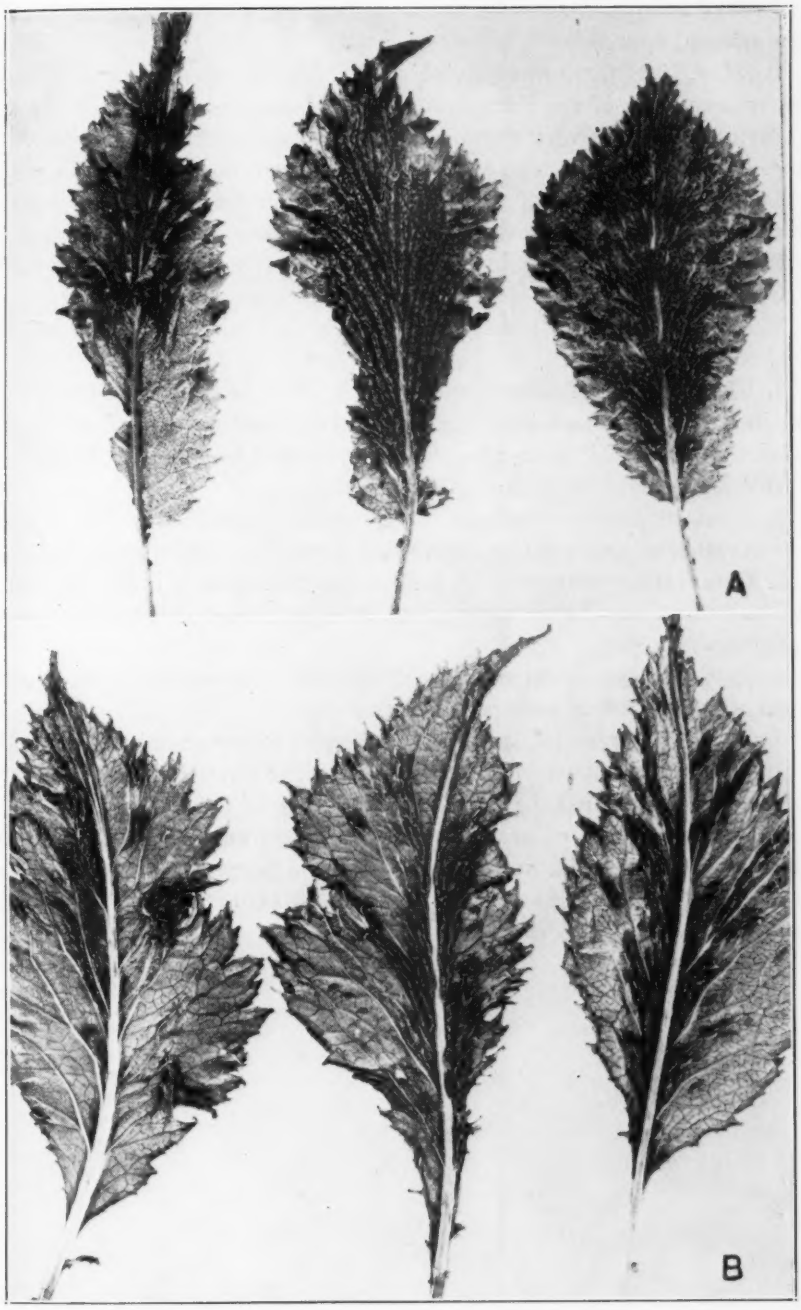

FIG. 1. Rasp leaf of cherry. A. Leaves from mazzard cherry seedling on which diseased buds had been placed. B. Leaves from Bing cherry on which diseased buds had been placed. The leaves on cion shoots showed even more distortion and leaf enations.

the axils of symptomless leaves borne on laterals of small branches that had some affected spurs.

By May, 1942, there was convincing proof in the nursery plots at Moscow that transmission of the virus had actually been accomplished in 1940–41, and symptoms were very pronounced in the second growing season. On Bing, on mazzard seedlings, and on Montmorency nursery trees inoculated in August, 1941, rasp-leaf symptoms showed on leaves growing near the point of insertion of diseased buds, although in some cases the buds had died during the winter. Trees inoculated with the (b) type bud wood showed no symptoms.

The writer's studies on rasp leaf of cherry in Idaho permit the following conclusions:

1. The rasp leaf of cherry, caused by a virus, is transmitted readily by bud inoculation; has an incubation period of less than 1 year (9 months) rather than 1 year (if spring grafted), as reported by Lott and McLarty[2] or 2 years, as reported by Bodine and Newton.[1]

2. Mazzard cherry seedlings, Bing cherry, and Montmorency cherry showed rather typical rasp-leaf symptoms 9 months after budding in August.

3. Observations supported by limited inoculation tests indicated that the rasp-leaf virus may be unevenly distributed in a host and probably moves rather slowly.

4. Rasp-leaf symptoms may be exhibited by leaves on terminal shoots of mazzard seedlings that have been bud-inoculated.

5. At present, rasp leaf is of no commercial importance in Idaho and no natural spread is known to have occurred. The devitalizing effect of rasp leaf, however, indicated the potential seriousness of the disease.

The widely separated areas in which rasp leaf has been reported—Colorado, British Columbia, and Idaho—raise the perplexing question of the origin and history of the virus.—EARLE C. BLODGETT, Idaho Agricultural Experiment Station, Moscow, Idaho.

BOOK REVIEW

FOSTER, ADRIANCE S. *Practical Plant Anatomy.* 155 p. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York. 1942. $2.50.

In this inexpensive little book the Plant Pathologist finds an up-to-date presentation of the essentials of the subject of Plant Anatomy that he is likely to need. The graduate student in Plant Pathology will likewise find this book helpful because it covers the fundamental aspects of the subject that he is expected to know, and does not require the study of a large volume of detail in order to obtain the desired information.

References covering the most important literature are listed at the end of each chapter. These provide a rapid means of locating classic as well as recent important contributions on various aspects of Plant Anatomy.—T. E. RAWLINS, Plant Pathology Division, University of California, Berkeley, California.

DEFINITIONS OF FUNGICIDE TERMS[1, 2, 3]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

TOXICITY: The ability of an agent to interfere adversely with the vital processes of an organism by physico-chemical means. TOXICANT: An agent capable of exhibiting toxicity; a poison.

FUNGICIDE: A chemical or physical agent that kills or inhibits the development of fungus spores or mycelium. A toxicant for fungi. BACTERICIDE: A toxicant for bacteria. INSECTICIDE: A toxicant for insects. NEMATOCIDE: A toxicant for nematodes.

PROTECTANT FUNGICIDE: An agent that protects the plant or plant part from infection by killing, or by inhibiting the development of fungus spores or mycelium that may arrive at the infection court (8, 14). In case of seeds—SEED PROTECTANT. PROTECTIVE VALUE: Measure of the ability of a protectant fungicide to prevent infection of the plant or plant part (7).

DISINFECTANT (general usage): An agent that kills or inactivates organisms on or within the plant or plant part, or the immediate environment. In case of seeds—SEED DISINFECTANT; soils—SOIL DISINFECTANT. A more precise and logical definition is to be preferred (1, 14), thus DISINFECTANT (restricted usage): An agent that frees from infection by destroying the pathogen established within the plant or plant part. Coordinate with this restricted use there is DISINFESTANT: An agent that kills or inactivates organisms present on the surface of the plant or plant part, or in the immediate environment. In the case of seeds—SEED DISINFESTANT; soils—SOIL DISINFESTANT.

ERADICANT FUNGICIDE: An agent applied to a plant, plant part or the environment to destroy fungi established within a given area of land or plant (8). Eradicant fungicides may function as disinfestants, disinfectants or both.

COLLOIDAL FUNGICIDE: A fungicide applied in the colloidal state, that is, made up of particles exhibiting Brownian movement which stay indefinitely dispersed and are within the range 0.2 to 0.005 μ in diameter (4).

FUMIGANT: A chemical toxicant employed in volatile form.

FUNGICIDAL (general usage): Pertaining to the ability of a fungicide to kill or inhibit the growth of fungi. FUNGICIDAL VALUE: Measure of ability of a fungicide to kill or inhibit the growth of fungi (7). FUNGICIDAL (restricted usage): Pertaining to the ability of a fungicide to kill fungi. In contrast (9) there is FUNGISTATIC: Pertaining to the ability of a fungicide to inhibit the germination of fungus spores or development of mycelium, while in continued contact.

PHYTOTOXIC: Pertaining to the property of killing or injuring plants, especially higher plants or their parts. Use in preference to PHYTOCIDAL.

[1] Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

[2] Definitions from here on may be generalized by substituting toxicant for fungicide.

[3] In 1939 a Committee on the Standardization of Fungicidal Tests was appointed by The American Phytopathological Society. The general aims of the Committee are to improve the methods of testing fungicides in the laboratory and field through cooperation and standardization.

Cooperative research is sponsored on the evaluation of various experimental designs, on the development of new methods, and in the correlation of field and laboratory results. Fungicide is defined in its broadest sense, and the methods include any phase in the testing other than chemical analyses. The procedure in standardizing is as follows: A certain method is brought to the attention of the Committee as being desirable, that is, it fulfills a definite need and has given satisfaction in at least several different laboratories. A mimeographed outline of the method is prepared as a "Tentative Method" and is made generally available. After a year or more of study and criticism by those interested, the tentative method, if found acceptable, is revised and presented in concise form to the Society for publication in Phytopathology as a "Recommended Method." Tentative methods not found acceptable are held for further study and modification. It is expected that the published Recommended Methods will be revised from time to time, if necessary.

The first Recommended Methods are appearing in this issue of PHYTOPATHOLOGY. Information concerning methods under current consideration either as Tentative or Recommended may be seen in the reports of the Committee to the Annual Meeting of the Society as published in PHYTOPATHOLOGY. Mimeographed copies of the Tentative Methods and reprints of the Recommended Methods may be obtained from the Committee Chairman (for address see Annual Report).

The Committee welcomes suggestions regarding new methods suitable for standardization.

DOSE, DOSAGE: Quantity of toxicant applied per unit of plant, fungus, lumber, soil, or special surface (6, 10). DOSE RATIO: Ratio between successively increasing doses (10). LD50: The median lethal dose, that is, the dose that kills 50 per cent of a large group of individuals (13). In case of spore germination tests—the dose that inhibits the germination of 50 per cent of the potentially viable spores (15). It is to be noted that a low LD50 VALUE indicates a high fungicidal value.

DEPOSIT: Quantity of dry fungicide[2] deposited on a unit area of plant, plant part or other surface, at any given application. The dried fungicide deposit remaining from the dose minus the run off. RUN OFF: Quantity of fungicide which runs off a unit area of plant, plant part or other surface during and immediately succeeding application. RESIDUE: Amount of dry fungicide remaining on a unit area of plant, plant part or other surface at any given time. DEPOSIT BUILDER: Material added to a fungicide to increase deposit rather than to increase tenacity.

ADHERENCE: Property of a fungicide to adhere or stick to a given surface. TENACITY: Property of a fungicide deposit or residue to resist removal by weathering (2). Tenacity is dependent on four major factors: adherence and insolubility of fungicide, nature of surface, and nature of weathering. TENACITY INDEX: The measure of tenacity. The ratio of quantity of fungicide residue per unit area of surface at the end of a given amount of weathering to that present at the beginning. STICKER: Material added to a fungicide to increase tenacity rather than to increase deposit.

SPREAD: Uniformity and completeness with which the fungicide deposit covers a continuous surface, such as a single leaf or seed. COVERAGE: Distribution of a fungicide deposit over a discontinuous area, such as leaves of a tree, or seeds. SPREADER: A material added to a fungicide to improve the spread of the liquid over a given area.

DILUENT: The component of a spray or dust that serves to reduce the concentration of active component and may aid in mechanical application, but does not directly influence toxicity. FILLER: A diluent in powdered form. CARRIER: A material serving as a vehicle for a fungicide.

SUPPLEMENT, *Syn.* ADJUVANT, AUXILIARY: A material added to a fungicide to improve some physical or chemical property (3, 11, 12). That is, a sticker, spreader, or deposit builder (see above), wetting agent, deflocculating agent, emulsifier, activator, or safener, but not the diluent. WETTING AGENT: A material that reduces the contact angle of a liquid on a given surface. Compare Spreader (3). Use wetting agent in preference to WETTER. DEFLOCCULATING AGENT: A material added to a fungicide suspension to delay sedimentation (12), in case of PROTECTIVE COLLOIDS, presumably by adsorption on the particles, and with DISPERSING AGENTS, presumably by dispersing aggregations of amorphous particles (11). EMULSIFIER: A material added to increase or stabilize the dispersion of one liquid within another. ACTIVATOR: A material added to a fungicide to increase either directly or indirectly its toxicity. SAFENER: A material added to a fungicide to eliminate or reduce phytotoxic effect. Used in preferene to CORRECTIVE.

AVERAGE PARTICLE SIZE: (a) The arithmetic mean diameter. (b) the diameter of particle of average surface; (c) the diameter of particle of average volume; (d) the diameter of particle of same specific surface as the material. It should be stated which of these four possible diameters (5) is actually measured.

FLOWABILITY: The property of flowing possessed by liquids, colloids, or dusts.

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THE SLIDE-GERMINATION METHOD OF EVALUATING PROTECTANT FUNGICIDES[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

The method of evaluating protectant fungicides in the laboratory by means of spore germination tests on sprayed slides has undergone considerable development since its first application over 30 years ago (28, 30). It is now the most extensively used of all methods for testing the fungistatic (15) or spore inhibiting ability of protective fungicides (14, 27). During recent years in particular the method has been subjected to a critical analysis (10, 11, 16, 20, 21, 25, 31) which has resulted in marked improvement; so that at present it may be considered a precise method fulfilling the demands of modern bioassay. The errors associated with spore germination tests of fungicides arise from two sources: biological and mechanical. A rigid control and standardization of the methods of producing and germinating the spores have greatly lessened the biological errors (11, 16, 20, 31), while recent developments in precision apparatus for applying sprays and dusts have materially reduced the mechanical variation (9, 10, 11, 16, 21).

The various specifications as outlined below are considered to embody the best current techniques. However, in order to meet the requirements and facilities of different laboratories it has seemed desirable for the present to offer, in certain instances, various alternate procedures. All of the factors specified have been found to be of importance and must be considered when using this method. Reference is given to the original papers for a detailed discussion of the various procedures, which should be consulted before attempting to employ this method.

GENERAL DESCRIPTION

The fungicide is applied to chemically clean glass slides by means of a precision technique such as a settling tower or horizontal sprayer. The former is suitable for sprays or dusts, the latter for sprays only. The deposition is regulated to give a series of dosages varying in geometric progression. The slides are allowed to dry and then placed in moist chambers. Fungus spores obtained under controlled conditions as regards species, strains, medium, age, temperature, concentration and stimulant if desired, are suspended in distilled water and pipetted onto the sprayed or dusted slides. The moist chambers are sealed with water and held at a temperature suitable for germination. In the case of water-soluble chemicals, the spores are added directly to the solution, and an aliquot then pipetted onto untreated slides. After a specified time the slides are placed under the low power of the microscope and the spores examined for germination. The percentage spores inhibited from germinating based on a specified count are plotted on logarithmic probability paper and the LD50 or LD95 obtained for comparing the relative merits of the different fungicides. Tenacity or rain resistance may be determined by subjecting the dried sprayed, or dusted slides to a laboratory "weathering" and comparing their toxicity to unweathered slides.

GLASSWARE

Cleaning. All glassware, slides, racks, moist chambers, test tubes, pipettes, beakers, etc., must be chemically clean. The use of potassium dichromate-sulphuric acid mixture is

[1] Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

satisfactory, but the glassware must be thoroughly rinsed at least five times in tap water followed by once in distilled water (13, 20). Chromium has marked fungistatic properties (15) and all must be removed. The glassware should be stored under dust-proof conditions.

Glass Slides. For ordinary purposes, 3 × 1 inch slides are used. Do not handle surface with fingers.

Special Slide Surfaces. Fungicides of low surface tension may require special type slides in order to control the area of fungicide in contact with the spores. Ordinary slides may be coated with cellulose nitrate (5, 8, 12, 13, 23, 24), or circles may be etched on the slides (26), cover glasses mounted on the slides (27), or cavity slides employed. Extreme care should be taken when attempting to delimit the spread of the drops that the materials used do not possess toxic or stimulant properties (13). Most of these special slides cannot be subjected to "rain" tests. However, if dilute cellulose nitrate, *i.e.*, 0.25 per cent in butyl acetate, is used to coat the slides they may be so exposed.[2]

Slide Racks. Racks to support the glass slides in the moist chambers may be made of solid glass rods bent into a U. Relatively unreactive sheet metal racks, such as aluminum, are also useful and more durable than glass.

Moist Chambers. Medium (200 mm. outside diam.), small (150 mm.), or large (250 mm.) moist chambers may be used. The chambers are inverted for use and a filter paper disc may be placed on the bottom to provide a background and to hold the slide rack in position. After placing the spore suspension drops on the slides the chambers are sealed with water. Do not place different fungicides, or different concentrations of the same volatile fungicide in the same chamber. Because of this, space is wasted in large chambers. A chamber set up with rack, slides, and drops of spores is illustrated in a paper by McAllan (13, p. 11).

APPLICATION OF FUNGICIDE

Sprays and Dusts. Sprays and dusts must be applied by means of a precision apparatus, either a settling tower (9, 21) or a horizontal sprayer (5, 10, 11, 21). The former is suitable for both sprays and dusts, the latter for sprays only. Settling towers are more precise and in general more elaborate than horizontal sprayers, while horizontal sprayers are more rapid for one or two test fungi and permit greater variability in dose ratio. Other methods of applying sprays such as the use of a micro pipette (26) or a solid glass rod with ground top (27) have been employed. Likewise, test tube dusters have been used commonly for applying dust fungicides (13). However, until these techniques can be demonstrated to be equal or better in precision than the settling tower or horizontal sprayer, they cannot be advocated for a standard method.

Settling Towers. Settling towers function on the principle of filling the tower uniformly with the spray or dust and then allowing it to settle on the glass slides. Fungicide suspensions contained in a beaker and constantly stirred are sprayed up into the tower through an atomizer nozzle located near the base of the tower. When the tower is filled with a fine suspension, spraying is stopped and a preliminary settling period allowed for the heavy drops to fall. The slides are then introduced at the bottom. After a given period of exposure, the slides are withdrawn and the tower evacuated by forced draft. The entire operation is then repeated and a series of fungicide deposits obtained on the slides according to the number of times they were exposed. It is necessary to standardize on a number of factors such as dimensions of tower, spraying pressure, time of spraying, time of preliminary settling, time of exposure, and positional effect, if any, of slides. The de Vilbiss No. 15 atomizer is considered standard for the settling tower and horizontal sprayer. A satisfactory settling tower of known precision is discussed in detail in a paper by McAllan (21). Every individual settling tower or horizontal sprayer when constructed must be calibrated; in addition, a special calibration may be necessary for fungicides of unusual physical nature.

Settling towers for dusts are modified in that a given quantity of dust is placed in a "gun" and shot upward into the tower by sudden release of air pressure. An apparatus that has given satisfaction in different laboratories is described (9) in detail.

Horizontal Sprayers. A horizontal sprayer is a stationary apparatus in which the fungicide is sprayed horizontally through an atomizer nozzle onto a facing glass slide held a set distance away. The distance is within the range 20 to 30 inches. The duration of spraying is precisely controlled by a cut-off valve or stop cock, and the deposition of fungicide is determined by the time of spraying. The apparatus is suitably housed to eliminate stray air currents, back pressure, and to maintain a high humidity. As in the settling tower, the fungicide is held in a beaker and continuously stirred, and the nozzle is a de Vilbiss No. 15. Sprayers of tested precision are discussed and illustrated (10, 11, 21).

Soluble Materials. Soluble materials not to be tested for tenacity may be applied by means of the test tube dilution technique (16). A series of dilutions is prepared in

[2] Unpublished data furnished by J. G. Horsfall.

test tubes and to a known quantity, usually 2 cc., of the chemical, 0.5 cc. of spore suspension is added and two individual drops of the resulting suspension pipetted onto the glass slides. If necessary, orange juice or other stimulant is added along with the spores. The concentration of chemical should be expressed as per cent or parts per million by weight of (a) toxic ion or radical, or (b) total fungicide. This technique is more rapid than the horizontal sprayer or settling tower, though less precise, and may be used for preliminary orientation tests of readily suspendable materials. Care should be taken to agitate the suspension of fungicide and spores before withdrawing the pipette sample.

Dose Ratio. Dosages, *i.e.*, settling tower exposure, spraying time, or concentration of soluble materials should be varied in geometric progression. Standard ratios of $\sqrt{2}$, *i.e.*, 1.414, 2, 4, $\sqrt{10}$, *i.e.*, 3.16 or 10 are recommended (15, 16). Sufficient dosages should be run so that at least three finite germination responses will be obtained (16). Steep toxicity curves will require small dose ratios, and flat curves, large ratios.

Deposition. Express deposition of sprays and dusts as milligrams or micrograms of (a) toxic ion or radical, or (b) total solids, deposited per square centimeter of surface (8, 11, 16).

STANDARDIZATION OF FUNGUS

Species. The following species have been used extensively and give satisfactory results (16): *Alternaria solani* (Ell. & Mart.) Jones & Grout, Delaware strain (16, footnote 5), *Glomerella cingulata* (St.) sp. & von S., *Macrosporium sarcinaeforme* (Cav.), and *Sclerotinia fructicola* (Wint.) Rehm. In addition, *Penicillium expansum* Link, and *Rhizopus nigricans* Ehr. + strain recently have been found suitable (15). All of these species may be used to test heavy metal and organic fungicides; only *Sclerotinia fructicola* is suitable for sulphur fungicides. In general, the use of at least two different species is recommended (16). Under the conditions specified below, the conidia of all six species should give a high percentage of germination in the controls, at least 95 per cent, except *Glomerella cingulata*, for which 90 per cent or higher should be obtained. Other species may be used provided they meet the requirements of reproducibility of results, ease of counting, and production of spores set forth (16, p. 70–72).

Culture Media. All six species may be cultured on test tube slants of potato dextrose agar (8, 15, 16, 20, 27) though oatmeal agar slants may be preferred for *Macrosporium sarcinaeforme* (3, 11).

Age of spores. The viability of most fungus spores changes markedly with age (4, 13). Spores from all species should be obtained from 7-day-old cultures (16, 20) except for the slower growing *Macrosporium sarcinaeforme*, where 14- to 21-day-old cultures are recommended (8, 11).

Density of Spore Suspension. It has been shown that the percentage germination response varies with the dose per spore and that there is a direct relation between the log. LD50 and the density of spore suspension (11, 16). Hence it is necessary to control the number of spores in suspension. The spore suspension concentration can be determined in a Fuchs-Rosenthal blood counting cell (20), and the final concentration should be adjusted to 50,000 spores per cc. This suspension will give about 35 spores per low power field (15× ocular, 16 mm. obj.) (16).

Application to Slides. The spore suspension is applied to the slides by means of a 1 cc. or 2 cc. pipette, care being taken to maintain the spores in suspension. Two pairs of drops may be placed on each slide, the four drops being in a staggered position (13). In the case of settling tower or horizontal sprayer, the first pair of spore suspension drops are of one fungus species and the second pair of another species, while with the test tube dilution technique the two pairs of drops may constitute two different doses of fungicide. The drop should be approximately 0.05 cc. in volume (11, 16). On plain glass the drops should spread to a diameter of about 10 mm. (16), and on cellulose nitrate to a diameter of about 7.5 mm. (11). Difficulty will be encountered with materials of low surface tension and special surfaces will be required as stated above. In all cases, the area of the drops should be maintained reasonably constant within a test. Extreme departures from the above diameters should be stated.

Temperature. The six fungi specified may be cultured and will produce a maximum of spores at temperatures from 20° to 25° C. (11, 16). As is well known, spores vary in their temperature requirements for germination (4), and it has been shown (3, 22, 31) that their resistance to a fungicide is greatest when the temperature is optimum. However, it has been found that within the range 15° to 27° C. either *Alternaria solani* or *Sclerotinia fructicola* which have respectively high and low optimum temperatures will rate compounds in the same order (31). Accordingly, the temperature range 20° to 25° C. is specified for spore production and germination. Where it is of interest to obtain greater information regarding temperatures the spores should be tested at two or more temperatures that vary by at least 10° C.

Stimulants. It has been demonstrated that much of the day-to-day variation or replicate test error may be due to the presence of variable amounts of water-soluble

nutrient derived from the cultures when procuring the spores (20, 26, 27). In such cases the spores should be obtained by a vacuum technique or they should be washed and centrifuged to rid them of the contaminating stimulant (20, 26). Washed spores of some species, particularly *Sclerotinia fructicola*, will not germinate readily in distilled water (20, 27), hence it is necessary to add a known quantity of stimulant. Ultra-filtered orange juice used at a strength of 0.1 per cent has proved very satisfactory for most species. A concentration of 0.316 is preferred for *Penicillium expansum* and *Rhizopus nigricans* (15). A frozen supply of the orange juice may be kept on hand (20). Extracts from commercial dried potato dextrose agar (27) and coenzyme R (6) have also been used. The amount and nature of stimulant employed should be stated. Stimulants have been shown to increase the resistance of the spores to chemicals (3, 15), hence will affect the comparisons of different species.

EXAMINATION FOR GERMINATION

Time. The spore may be examined for germination after 20 to 24 hours (18, 31). However, it has been found that a given fungus may rate fungicides in a different order at different periods of time, though usually no further change may be expected after 50 hours (31). Hence, when it is of interest to obtain greater information the spores should be examined at 6 hours and at 24 hours.

Magnification. A magnification of 100× is recommended for counting spores of *Macrosporium sarcinaeforme*, and 150× for *Alternaria solani*, *Glomerella cingulata*, and *Sclerotinia fructicola* (16). A cover slip is not required under ordinary circumstances.

Number of Spores. One hundred potentially viable spores should be counted per deposit or concentration of each compound, and for each species, provided it has been established that the error for replicate counts in the given laboratory is approximately that of the binomial distribution (17, 20). Otherwise, a replicate count of a second 100 spores is desirable. The count of 100 spores will consist of approximately 50 spores from each of the two spore suspension drops. Potentially viable spores are those that would germinate under the normal conditions of the control. A simple correction for potentially viable spores in the case of high germinating controls is as follows: count as many extra spores as the control is short of 100, and deduct this number from the ungerminated results. For example, if control gives 97 per cent germination, count a total of 103 germinated and ungerminated spores and subtract 3 from the ungerminated results. A more precise correction for lower controls is: corrected per cent germination = observed per cent germination × 100/control per cent germination (31). The counting of germinated and ungerminated spores is greatly expedited by means of a double hand tally counter, which may be operated by one hand and will record both classes.

Length of Germ Tube. The spore is arbitrarily defined as germinated if the length of the tube exceeds half the minor diameter of the spore. An approximation of the average germ tube length in microns for each count of 100 spores may be recorded. However, since germ tube length and percentage of germination is usually highly correlated, little is gained by measuring germ tubes (14).

Clearing the Microscopic Field. In the case of heavy deposits of fungicides, it is frequently difficult to see the spores. Copper fungicides may be readily cleared by the addition of a drop of concentrated acid. Organic solvents are sometimes successful with organic materials. A solution of 50 per cent chloral hydrate and 0.02 per cent safranine has been found fairly successful for staining spores of *Sclerotinia fructicola* and rendering them more visible in suspensions of sulphur fungicides.[3]

Recording Results. In order to conform to standard procedures used in toxicity measurements of insecticides, drugs, etc., the percentage of spores inhibited from germinating should be recorded (14, 33).

COMPARISONS

Dosage-response or Toxicity Curve. In most toxicological data it has been found that the distribution of individual lethal doses tends to be symmetrical when plotted against the logarithm of the dose (29, 33). Because of this symmetry, if the toxicity curve is plotted on logarithmic-probability paper a straight line will result. Thus the per cent spores inhibited from germinating should be plotted against the dose or concentration of the fungicide on the logarithmic-probability paper[4] and the best straight line drawn through the points giving greatest weight to those nearest the center (33). However, in some cases, the points will have a definite trend away from a straight line, either toward a curve concave upwards, convex upwards, or sigmoid (16), or of a more irregular shape (3). These departures from the normal straight line should be substantiated by replication. In such cases "broken" straight lines must be plotted. With the data plotted as straight lines it is possible to compare fungicides on the basis of dose for equivalent inhibition of germination or control (3, 16, 33).

[3] Unpublished data furnished by S. E. A. McCallan.

[4] Codex Book Co., Inc., Norwood, Massachusetts.

Unit of Comparison. The common unit of comparison is the LD50 or dose inhibiting the germination of 50 per cent of the spores (11, 16, 19, 29, 33). The LD50 can be most precisely determined for a given spore count (29), and is best adapted for comparing straight line or concave type toxicity curves and for materials having the same slope (16). The LD95 is more practical for comparing fungicides having curves of the convex or sigmoid or irregular type and dissimilar slope (3, 16). When the LD95 is used it should be so stated.

Standard Fungicide. "Standard Laboratory Bordeaux Mixture" may be employed as a basis for general comparisons and for determining "Bordeaux Coefficients" (2).

Replications. Because of day-to-day variations in the resistance level of the spores, tests should be replicated on different days using different lots of spores, rather than at the same time (11, 16, 17, 20). It is preferable to test with several different fungi twice rather than one fungus a number of times (16).

Error Term. In making statistical comparisons of the LD50 or LD95 of a different fungicide, the compound × replicate test interaction ordinarily will be used for the error term (16). For a further discussion of statistical treatment see (16, p. 74).

DETERMINATION OF TENACITY

It is desirable to determine the tenacity of fungicides after they have been subjected to laboratory weathering tests. The tenacity evaluated is dependent not only on the physical chemical properties of the fungicide, namely adherence and insolubility, but also on the two basic variables, nature of surface sprayed and method of weathering.

Nature of Surface. Glass (14, 19, 26) and cellulose nitrate surfaces (7, 23) commonly have been used. Information is not available as to which more closely resembles leaf surfaces in general or any leaf in particular.

Method of Determination. There are two basic methods of weathering to determine tenacity: (a) "artificial rain" and (b) immersion. The "artificial rain" may be applied by allowing water under pressure to flow upward through a rose-type nozzle and thence downward by gravity onto slides oscillated or rotated beneath (19), or it may be applied directly through a horizontal sprayer (24). The immersion method may consist simply of suspending the slides in a beaker of water for a short (32) or long (23) period of time, or of shaking the slides during or after immersion or both (7, 26). It would appear that "artificial rain" more closely simulates natural weathering, but immersion is simpler and more rapid. Limited tests indicate that these methods differed but little in their ability to remove the fungicide deposit (7). The procedure employed should be stated in detail.

Tenacity Index. The tenacity index is the ratio of active fungicide remaining after rain tests to that originally present (1). It may be determined by customary chemical analyses or by spore germination tests (7). In the latter case spores are germinated on unweathered slides and on a comparable set of weathered slides. The tenacity index is then derived (7) as follows: $\frac{\text{LD50 for unweathered slides}}{\text{LD50 for weathered slides}}$. The tenacity index may also be expressed on the basis of the LD95 and should be so stated.

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STANDARD LABORATORY BORDEAUX MIXTURE[1]

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

In the laboratory evaluation of fungicides it is necessary to employ a standard fungicide (a) as a check on the reproducibility of the technique, (b) as a basis of comparison for new compounds, and (c) under specified conditions, as a means of adjusting day-to-day variations due to changes in the resistance level of the spores (2, 4, 8). It is typical of bioassay in general, that each population will vary from day to day. Here each population of spores will vary in its resistance to fungicides. Controlled conditions have reduced but not eliminated this day-to-day variation (2, 4, 5, 7). Where a suitable standard is carried with each test, it is possible to rate each compound in terms of the standard and thus correct much of the day-to-day variation or replicate test error (2, 4, 8).

A standard fungicide should fulfill certain requirements. It should be well known, readily obtainable, easy to prepare, of known composition, stable and of medium toxicity (2). In addition, for a precise comparison, it should be of essentially similar chemical composition, and have a toxicity curve of similar slope to the "unknown" fungicides with which comparisons will be made (4). It is apparent that no single material can fulfill all of these requirements and function as a universal standard. It has been shown that day-to-day variation can be adjusted effectively only when standard and unknown are of similar chemical composition and slope (1, 4). Hence, a general standard may be selected to check on reproducibility of technique and as a basis of comparison for new materials, but it will have only limited application in correcting day-to-day variation (4).

Bordeaux mixture, the most important member of the most important group of fungicides, the copper compounds, is presented (2) as a suitable standard laboratory fungicide under the conditions above outlined.

PREPARATION OF STANDARD LABORATORY BORDEAUX MIXTURE

The variability in Bordeaux Mixture is eliminated by standardizing the composition of the ingredients, the method of preparation and the temperature. Stock solutions instead of solids are used to insure rapid and uniform reactions. Temperature is held constant between 20° and 25° C. and reagent quality ingredients free of carbonates are employed (2).

Stock Copper Sulphate. A 3.928 per cent solution of reagent quality $CuSO_4 \cdot 5H_2O$ (1.0% Cu) is made up in distilled water.

Stock Lime Water. A saturated lime water solution is prepared by suspending an excess of reagent quality hydrated lime in boiled distilled water. Agitate thoroughly to obtain saturation. Let settle. Keep in siphon bottle protected from CO_2 with soda-lime tower.

Standard Bordeaux. The volume ratio of stock copper sulphate and lime water should be 1 to 12 (mol. ratio 1 Cu to 1.65 Ca). The stock copper sulphate should be added to the stock lime water while stirring. The quantities in cc. of stock copper sulphate to be added to stock lime water and diluted to obtain varying percentages of copper are as follows:

[1] Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

Final volume in cc.						Per cent copper
250		500		1000		
Copper	Lime	Copper	Lime	Copper	Lime	
5	60	10	120	20	240	0.02
.....	.....	5	60	10	120	0.01
.....	.....	.....	.....	5	60	0.005

DEPOSITION AND LD 50 VALUES FOR STANDARD BORDEAUX

In comparing the Bordeaux standard and "unknown" fungicides, the materials should be applied by means of a standard precision technique such as the settling tower (6) or horizontal sprayer (2). The approximate deposition of copper in microg. per sq. cm. to be expected from a Standard Bordeaux containing 0.005 per cent Cu for the two techniques is, respectively, 0.05 per one exposure (settling tower) and 0.03 per second of time (horizontal sprayer). The LD50 values (8) for spores of *Alternaria solani*, *Glomerella cingulata*, *Macrosporium sarcinaeforme*, and *Sclerotina fructicola* tested under the standard conditions (2, 3, 4, 5, 6, 7, 8) of the slide-germination technique should range from approximately 0.2 to 0.5 micrograms copper per sq. cm.

DETERMINATION OF BORDEAUX COEFFICIENT

The toxicity of copper compounds with toxicity-curve slopes similar to Standard Bordeaux Mixture may be expressed as Bordeaux Coefficients (2) according to the formula:

$$\text{Bordeaux Coefficient} = \frac{\text{LD50 deposition of copper in Standard Bordeaux}}{\text{LD50 deposition of copper in Fungicide X}}.$$

The coefficients thus obtained will vary from test to test or day to day less than the actual LD50 values. For example in 10 tests of red cuprous oxide the coefficient of variation for the LD50 values was 45.4 but expressed as Bordeaux Coefficients it dropped to 23.9 (2). Hence the day-to-day variability was adjusted to one-half by the use of a suitable standard.

The toxicity of fungicide materials markedly unlike Bordeaux Mixture in composition and slope of curve may be expressed as Bordeaux coefficients on the basis of total solids in each, in order to make general comparisons with the Standard. However, in such cases as stated above, effective correction for day-to-day variation is not likely to result.

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A POSSIBLE REPRINTING OF SACCARDO'S SYLLOGE FUNGORUM

The Alien Property Custodian has recently announced (Science 97: 303–4. 1943) that many technical books and sets of books of Axis origin are available for republication. The procedure to be followed in obtaining necessary licenses and other details is given and it is clear that every encouragement will be given to bring about prompt reproduction of books of this kind.

To mycologists and plant pathologists this announcement immediately suggests the possibility of the reproduction of Saccardo's classical *Sylloge Fungorum* in usable form. This compendium of mycological descriptions is a "sine qua non," and the comparatively few sets now in use in the Americas are showing the effect of much use. Additional copies have been practically non-existent heretofore, only occasional sets appearing on the market at rare intervals and at exorbitant prices. There are undoubtedly many institutions as well as individual mycologists and plant pathologists who will welcome an opportunity to purchase a set of Saccardo. Even those libraries now possessing the work will likely desire an additional set to relieve the wear and tear on the original.

It has been ascertained that a satisfactory reproduction of the 25 volumes can be produced. If 100 subscriptions are obtained by September 1, the complete set of 25 volumes can be obtained at $200.00 per set; if 300 subscriptions are obtained, the price will be $150.00 per set. These prices are based on an offset edition, with the type block photographically reduced ten per cent.

In order that the undersigned may obtain an idea of the number of prospective purchasers, interested mycologists are requested to send in tentative subscriptions and to interest their respective institutions in doing likewise.

JOHN A. STEVENSON,
Bureau of Plant Industry, Soils
and Agricultural Engineering,
Beltsville, Maryland.

SUMMER MEETING OF THE UPPER MISSISSIPPI VALLEY PLANT PATHOLOGISTS

A summer meeting of plant pathologists of the North Central States has been scheduled for August 12 and 13 at Purdue University, Lafayette, Indiana. Headquarters for the meeting will be at the Purdue Memorial Union Building on the campus where room reservations may be made. The program will be organized about the solution of problems and the exchange of ideas pertinent to the maximum production of food and fiber in the war emergency. The war committee and the national officers of The American Phytopathological Society are scheduled to meet with the Upper Mississippi Valley group.